Universidade de Aveiro Departamento de Biologia
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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia e Ecologia das Alterações Globais, realizada sob a orientação científica do Professor Doutor Carlos Manuel Martins dos Santos Fonseca, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro e coorientação científica do Professor Doutor Emílio Javier Virgós Cantalapiedra, Professor Titular da Universidad Rey Juan Carlos (Madrid, Espanha), e da Professora Doutora Mónica Sofia Vieira da Cunha, Professora Auxiliar Convidada da Faculdade de Ciências da Universidade de Lisboa.

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Dedico este trabalho à Biodiversidade,

Aos que procuram cuidar da ‘Casa Comum’,

Àqueles que tombaram quando protegiam a vida selvagem…

“… não basta pensar nas diferentes espécies apenas como eventuais «recursos» exploráveis, esquecendo que possuem um valor em si mesmas.”

Franciscus *in* ‘Laudato Si’

o júri

presidente

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palavras-chave

Herpestes ichneumon; biometrias; dimorfismo sexual; competência imune; condição corporal; ecologia trófica; ecologia reprodutiva.

resumo

O sacarrabos (*Herpestes ichneumon* Linnaeus, 1758) é um carnívoro que, na Europa, se encontra restrito à Península Ibérica e cuja distribuição em Portugal esteve mais confinada a Sul do Rio Tejo, até meados dos anos 90 (séc. XX). Durante as últimas décadas, esta espécie tem vindo a expandir-se do interior para o litoral e de Sul para Norte. Algumas populações são já encontradas na região biogeográfica Atlântica, onde encontram novas condições ecológicas, climáticas e de *habitat*. O sacarrabos é conotado negativamente pela comunidade de caçadores, que o imputa de depredar maioritariamente as espécies de caça menor com maior interesse económico, como o coelho-bravo, a perdiz e os seus ovos, bem como de gerar ninhadas múltiplas e numerosas. Na verdade, estas preocupações carecem de confirmação científica. Os aspetos ecológicos conhecidos em sacarrabos devem-se principalmente aos trabalhos científicos desenvolvidos nas populações da região de Doñana (Espanha) ou de Israel. Os estudos sobre a ecologia das populações deste carnívoro em território português são escassos ou restritos a algumas regiões. A ecologia do sacarrabos à escala biogeográfica, cobrindo diferentes padrões ecológicos, climáticos ou de pressão antrópica é desconhecida. Este trabalho pretende contribuir para o conhecimento dos aspetos ecológicos fundamentais, tróficos e reprodutivos do sacarrabos, quer nas áreas de distribuição histórica, como nas zonas de expansão e recentemente colonizadas. Foram assim amostrados 678 sacarrabos provenientes de atropelamentos acidentais (33), ações de correção de densidade de predadores e atos legais de caça (645) no território de Portugal continental. Neste contexto, os principais objetivos deste trabalho compreendem: 1) a descrição e análise dos parâmetros biométricos; 2) a determinação e a análise dos fatores que regulam a competência imune e a condição corporal; 3) a identificação das principais presas e a avaliação da ecologia trófica, entre géneros, classes etárias, estações do ano e regiões, moldados pelas diferentes características ambientais, condições climáticas e pressões antrópicas; 4) a identificação dos períodos reprodutor, de gestação, de lactação, com determinação do tamanho da ninhada e análise dos padrões reprodutivos. Os resultados apurados permitiram registar, pela primeira vez, dimorfismo sexual no tamanho corporal de sacarrabos em território lusitano, confirmando-se que os machos são maiores que as fêmeas, além de se apurarem diferenças biométricas marcadas entre regiões, que se sugere serem reguladas pela seleção sexual, disponibilidade alimentar, pressão humana e pelos diferentes padrões de utilização do *habitat*. Neste trabalho, foram também reveladas, pela primeira vez, as médias dos pesos e das principais medidas corporais de juvenis tipo I, tipo II e de sub-adultos. Neste estudo, foram ainda identificadas variações na competência imune ao longo do ano, que aparenta ser maior nos machos, justificadas pelo comportamento reprodutivo, parasitismo, ou enfermidades. Foi detetada uma relação estatísticamente significativa entre a competência imune e a condição corporal, que difere também entre géneros e idades, devido à ecologia trófica e reprodutiva. A análise da dieta permitiu observar que as espécies de caça menor representam menos de metade do espectro alimentar consumido. Neste estudo foram determinados os tamanhos médios (3) ($\bar{x}$=2.75), a extensão (1 a 4) e a moda (3) das ninhadas. Identificaram-se os períodos férteis dos órgãos reprodutores, de gestação e de lactação, que se encontram associados ao aumento do fotoperíodo e da produtividade primária, e que estão confinados à época que inicia no auge do Inverno e que decorre até ao início do Verão. Por fim, nos machos verificou-se existirem conflitos na alocação de recursos à reprodução *versus* competência imune ou condição corporal. Com as informações extraídas desta investigação, espera-se contribuir para a criação de uma base de conhecimento para futuros estudos científicos relacionados com a sanidade, ou com as alterações climáticas, e para a reavaliação do estatuto cinegético e melhoria da gestão do sacarrabos em Portugal.

keywords

Herpestes ichneumon; biometrics; sexual dimorphism; immune competence; body condition; trophic ecology; reproductive ecology.

abstract

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a carnivore that in Europe is restricted to the Iberian Peninsula and whose distribution in Portugal was previously confined to the South of the Tagus River until the mid-1990's. During the last decades, this species has been expanding geographically, from inland to the coast and from South to North. Some populations of Egyptian mongoose are already found in the Atlantic biogeographical region, where they find new ecological, climate and habitat conditions. The Egyptian mongoose is negatively perceived by the hunting community who believes it to predate mostly on small game species with great economic interest, such as the European rabbit, the red-legged partridge and its eggs, and to generate multiple and numerous litters. However, these concerns lack scientific clarification. Ecological aspects of Egyptian mongoose are known mainly from scientific work carried out on the population of the Doñana region (Spain) or Israel. Studies on the ecology of the Portuguese populations of this carnivore are scarce or restricted to certain geographic areas. The ecology of the Egyptian mongoose on a biogeographic scale, covering different ecological and climatic patterns or human pressure is unknown and lacks comprehension and scientific evidence. This work aims to contribute to the knowledge of the fundamental, trophic and reproductive ecological aspects of the Egyptian mongoose, both in its historical distribution areas, and in the expansion and recently colonized areas. In this study, 678 mongooses were sampled from accidental road-kills (33), density correction actions and legal acts of hunting (645) in the territory of mainland Portugal. In this context, the main objectives of this study include: 1) description and analysis of biometric parameters; 2) determination and analysis of the factors that regulate immune competence and body condition; 3) identification of the main prey and evaluation of its trophic ecology, according to gender, age classes, seasons and regions, shaped by different environmental characteristics, climatic conditions and human pressures; 4) the identification of reproductive, pregnancy and lactation periods, determination of litter size and analysis of reproductive patterns. The results obtained indicate for the first time sexual dimorphism in body size in the Egyptian mongoose populations of the Portuguese territory, in which males are larger than females, in addition to clear biometric differences between regions. The reported variations are suggested to be regulated by sexual selection, food availability, human pressure and by different habitat use patterns. For the first time, the average weight and main body measurements of juvenile type I, type II and sub-adults were determined. Variations in immune competence were identified throughout the year, which was higher in males, justified by the reproductive behavior, parasitism, or diseases. A statistically significant relationship between immune competence and body condition was detected, which also differs between genders and ages due to trophic and reproductive ecology. Diet analysis indicates that small game species represent less than half of the consumed food spectrum. In this study the average sizes (3) ($\bar{x}$=2.75), range (1 a 4), and mode (3) of the litters were also determined. Additionally, the active periods of reproductive organs, pregnancy and lactation were identified, which are associated to the increasing photoperiod and to primary productivity, starting at the peak of winter and elapsing until the beginning of summer. Finally, we found male-specific conflicts in resource allocation between mating effort/parental investment and body condition, as well as with immune function, supporting the notion of an energetic trade-off and fitness cost associated with reproductive performance. With the information drawn from this research, we hope to shed light on mongoose's bio-ecology and to create a framework for future research, either related to health or climate change. This work is also a contribution for the reassessment of the hunting status and improvement of the Egyptian mongoose management in Portugal.

Declaro que esta tese é integralmente da minha autoria, estando devidamente referenciadas as fontes e obras consultadas, bem como identificadas de modo claro as citações dessas obras. Não contém, por isso, qualquer tipo de plágio quer de textos publicados, qualquer que seja o meio dessa publicação, incluindo meios eletrónicos, quer de trabalhos académicos.

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CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

It is now widely recognized that global changes and biodiversity are interconnected (Convention on Biological Diversity, 2012). Biodiversity is affected by these changes, with subsequent negative consequences for humans (Parry *et al*., 2007; Convention on Biological Diversity, 2012). However, biological diversity contributes to mitigation and adaptation to global changes through the wide range of ecosystem services that it supports (Parry *et al*., 2007; Convention on Biological Diversity, 2012). The conservation and sustainable management of biodiversity are thus essential to face such system deviations (Convention on Biological Diversity, 2012). Habitat adjustments and species distributions have cumulatively been reflecting the effects of global change (Convention on Biological Diversity, 2012), allowing some opportunistic species to occupy new niches (Logan, 2007). Opportunistic species are less sensitive to resource limitations and population sizes can augment along with favorable events (Levinton, 1970). The population size increase of opportunistic species concurs with the decline of species with more specific ecological niches, potentially threatening the ecological balance of an ecosystem and, thus, jeopardizing biodiversity (Dutra *et al*., 2012).

Biological encroachments are a major threat to human health, agriculture and ecosystem sustainability (Hampton *et al*., 2004), resulting in the reduction or extinction of native species (Santos *et al*., 2007). Once established, opportunistic species may cause an irreversible event with serious consequences on the environment in new distributions.

Ecological monitoring allows the application of more effective and specific actions in the management of wild populations of opportunistic species. The identification of the resource use patterns of each species is a fundamental step to unravel how the ecosystem is organized, the pattern of species coexistence and the niche structure (Tokeshi, 1999). In this context, the study of trophic ecology is fundamental, because the diet has a crucial dimension on ecological niche (Krebs, 1989). Knowledge about the diet contributes to the understanding of dietary preferences and the conditions that allow the expansion and the success of a species (Rosalino *et al*., 2009). Reproductive ecology is another factor to take into consideration, in order to understand the effect of management actions on litter size, the duration of the fertile period during the year, or body weight of females in the different

periods (Abe *et al*., 2006). Animal reproductive performance may also be an indicator of environmental health.

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is one of the most abundant, natural, mammalian colonizers of mainland Portugal (see Gaubert *et al*., 2011). The mitochondrial diversity of the Egyptian mongoose across the Iberian Peninsula, where the species is currently expanding north-west, confirmed its long-term presence in the region, evidenced by haplotype segregation and strong differentiation between Iberian and North African populations (Barros *et al*., 2016*a*). Evidence of substructure and significant genetic differentiation within the Northern and Southern population divided by Tagus River were recorded, suggesting that the river acted as a semi-permeable barrier, possibly leading to genetic differentiation between the two populations (Barros *et al*., 2016*b*). The distribution area of this carnivore was once concentrated in the south of the Portuguese territory (Borralho *et al*., 1996). However, the species has been expanding to the north, and from the inland to the coast, a phenomenon that still is in progress (Barros & Fonseca, 2011; Barros *et al*., 2015). Currently, it is already present on Atlantic biogeographical region, where it finds new environmental conditions (Figures 1.1 and 1.2).

Figure 1.1. Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) specimen.

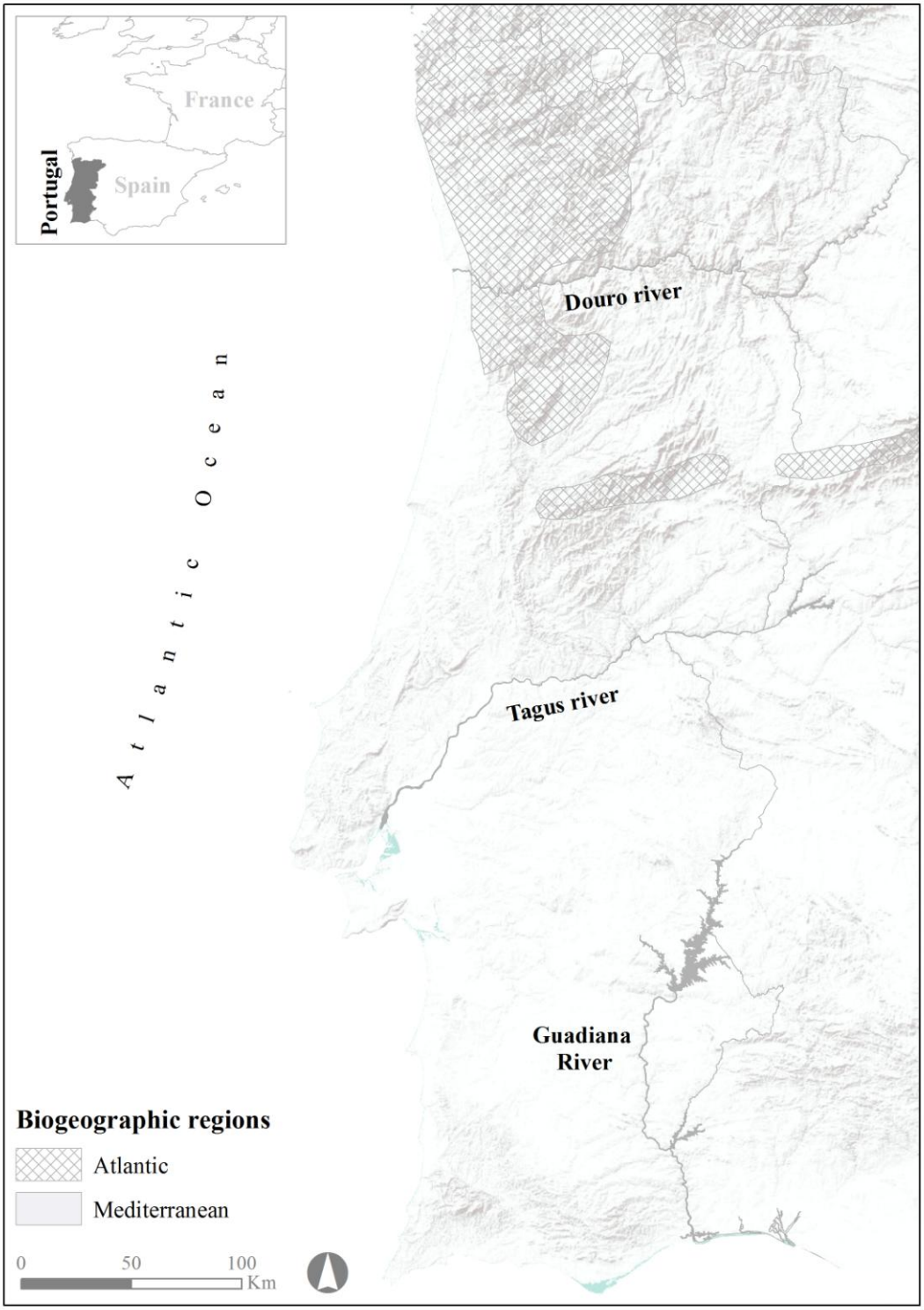

Figure 1.2. Representation of the biogeographic regions within mainland Portugal (adapted from Rivas-Martínez *et al*., 2004).

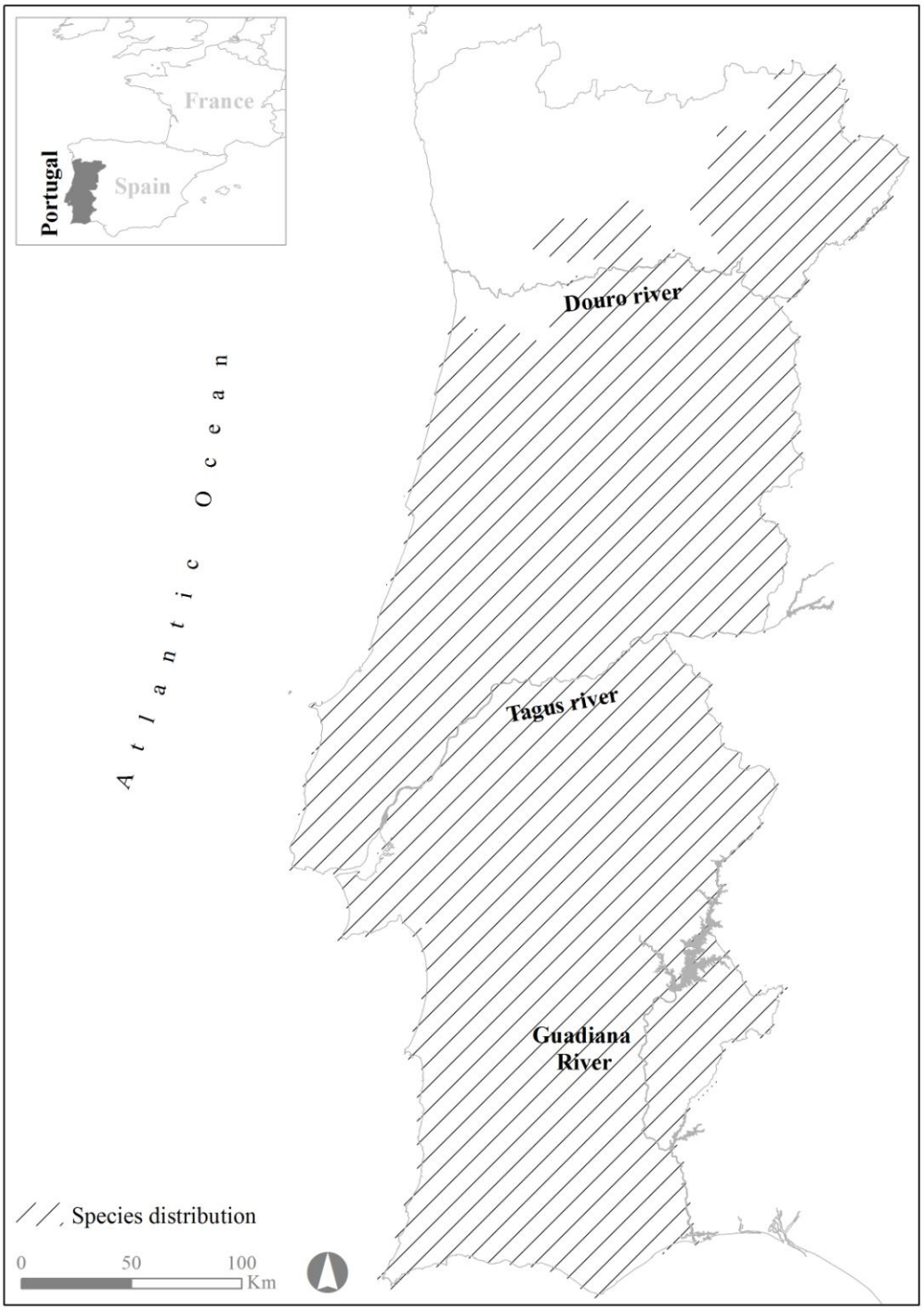

Figure 1.3. Current distribution of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal. (data from the present study and data adapted from Barros *et al*., 2015).

Over the past 80 years, it is estimated that the Egyptian mongoose has undergone successive expansions and contractions in its distribution area in Portugal (Barros & Fonseca, 2011). During the 1930s, the loss of suitable habitat, thick Mediterranean shrubland maquis, due to increased cereal production, and changes in land use jointly promoted the contraction of the occupancy area of this species (Barros & Fonseca, 2011). On the other hand, the recent abandonment of agricultural land, rural depopulation (Borralho *et al*., 1996), and the variation in average temperatures and annual rainfall (Barros *et al*., 2015) have cumulatively contributed to the recent occupation of new territories.

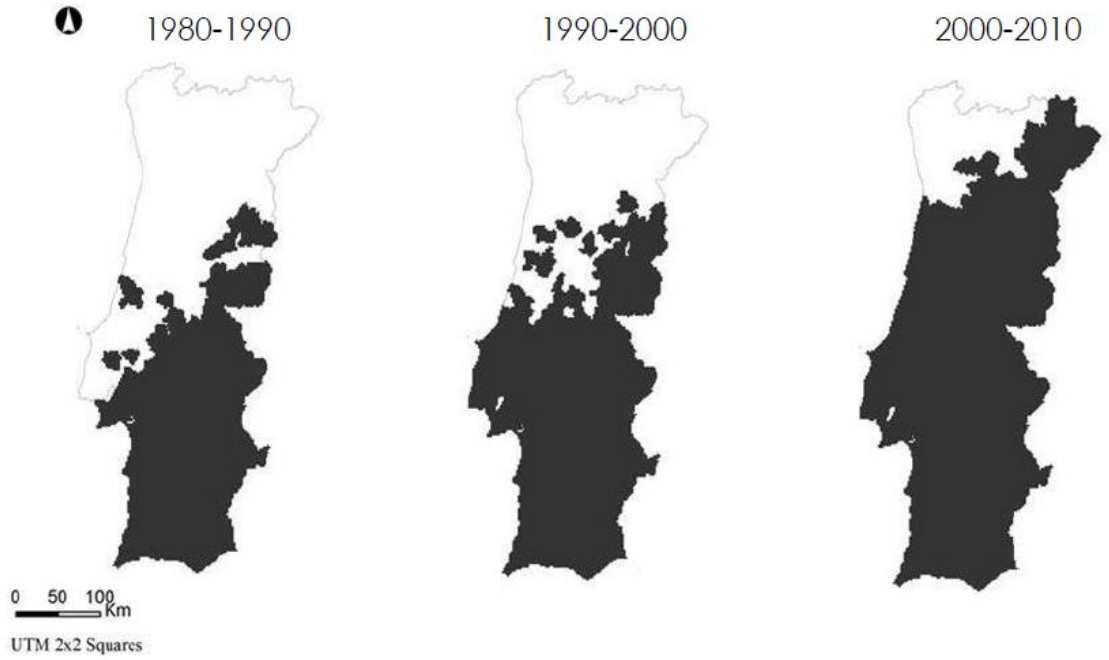

Figure 1.4. Scope of the expansion of the Egyptian mongoose (*Herpestes ichneumon*) in the last 30 years in Portugal according to inquiries and registries (data adapted from Barros *et al*., 2015).

The conservation status of Egyptian mongoose is Least Concern (LC) in Portugal (Cabral *et al*., 2005), as in global IUCN Red List (Do Linh San *et al*., 2016). According to the "Decreto-Lei nº 227-B/2000" and "Decreto-Lei nº202/2004" with the new wording given by "Decreto-Lei nº 201/2005", it is listed as a game species in the Portuguese territory, where a large number of Egyptian mongoose are culled in each hunting season (ICNF, *unpublished data*). This species in Portugal is negatively perceived by hunters, who

consider it responsible for the predation of the most important small game species, which include the European rabbit (*Oryctolagus cuniculus*) and the red-legged partridge (*Alectoris rufa*). Scientific studies point to it being an opportunistic and generalist predator, capturing more abundant, available and vulnerable prey, according to occasion (Delibes *et al*., 1984; Palomares & Delibes, 1998). Periods of activity of this carnivore coincide with periods of resting of most prey species (Palomares & Delibes, 1992*a*), thus being more active during the day, and inactive from sunset until an hour or two before dawn, with two peaks of activity registered, one in the morning and another in the afternoon (Delibes & Beltran, 1985). Prey are located and captured by an intense exploration activity, on the ground or by digging frequently (Palomares & Delibes, 1991; Palomares & Delibes, 1998). Typically, the Egyptian mongoose avoids open areas and prefers dense vegetation, with great cover and protection, where exploratory activities predominate, in habitats such as riparian corridors in Mediterranean environments, brambles and xerophytic scrubland, which are very productive habitats (Palomares & Delibes, 1990; Palomares & Delibes, 1991*b*; Palomares & Delibes, 1993; Virgós, 2001; Santos *et al*., 2011).

Figure 1.5. Mediterranean maquis - characteristic habitat of the Egyptian mongoose (*Herpestes ichneumon*).

Shrub cover over their burrows has three main functions: improvement of the microclimate, protection from trampling by larger species and reduction of the risk of predation (Blaum *et al*., 2007). Egyptian mongoose use latrines next to resting sites and increase their scent marking behavior when they are mating, in January and February (highest peak), and in September and October, when family groups are larger (Palomares, 1993). During the breeding season, behaviors and olfactory communication reach their peak (Ruiz-Olmo, 1997).

Up to the present moment, this species has not been subject to thorough investigation in Portugal, particularly on its fundamental and reproductive ecology. There is a study concerning biometrics that is restricted to the Serra de Grândola region (Rosalino *et al*., 2005). At national scale, there is only one study of trophic ecology that did not contemplate expansion regions (Rosalino *et al*., 2009). Most of the knowledge on Egyptian mongoose biology comes from studies conducted on populations from southwestern Spain (eg. Delibes *et al*., 1984; Palomares & Delibes, 1992*b*; Palomares & Delibes, 1993) and Israel (Ben-Yaacov & Yom-Tov, 1983). The work enclosed in this dissertation aimed to collect data regarding trophic ecology, reproductive biology and the phenotypic and morphological characteristics of the Egyptian mongoose, through a widespread sampling effort. This effort succeeded to cover the main habitats of the two biogeographic regions of the Iberian Peninsula where the species is distributed, including its previous distribution, and the newly occupied territories. These ecological data are herein combined with the specimen region, bioclimatic variables, habitat type, land use, or seasonality. Six hundred and seventy-eight wild Egyptian mongoose specimens were collected from 13 out of 17 districts of mainland Portugal where the species is distributed. Their origin was from hunting activities (645 specimens) and from accidental road kills (33 specimens), in compliance with legal requirements and with permits from the competent authorities. Depending on the origin of the harvest, each sample was assigned to a particular region, north or south of the Tagus River, which may be considered a geographical barrier for the species, bearing in mind that, until the 90s (of the XX century), the distribution of the Egyptian mongoose was more concentrated in the south of the Tagus River (Borralho *et al*., 1996; Barros & Fonseca, 2011; Barros et al., 2016*b*). Regarding land cover and habitat characteristics of each region, flora found in the north is mainly characterized by monoculture of *Eucaliptus* sp., which replaced a large part of the areas occupied by *Pinus*

pinaster, *Quercus robur*, *Salix* sp. or *Alnus glutinosa* (Alves *et al*., 2009). In the south, the prevailing flora consists of *Quercus suber*, with well-populated *Olea europaea* and *Quercus ilex* areas (Alves *et al*., 2009). In contrast with the south, the north is also characterized by higher human density, more kilometers of road, much more fragmented and altered by human action habitats, greater number of ridges with higher altitude, more rocky areas, more hydrography, lower temperatures, higher rainfall (see CIGeoE, 2016; INE, 2016) and lower densities of prey target species (Instituto da Conservação da Natureza e das Florestas, *unpublished data*; Loureiro *et al*., 2008). To compare the mongoose population from historic distribution (South of Tagus River) with the population in expansion (North of Tagus River) a battery of environmental, climatic and ecological variables was provided to complement study area information and to give insights on the region differences that each population faces. These variables are the number of hectares of each habitat type (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests and agriculture areas) retrieved from Corine Land Cover (2006). Mean altimetry value (data SRTM, NASA, resolution of 30 meters) gathered using the ASTER Global Digital Elevation Model platform (http://gdem.ersdac.jspacesystems.or.jp/) (ASTER, 2016). Number of inhabitants per km^2 in each grid using data from Eurostat per kilometer (http://epp.eurostat.ec.europa.eu/) (European Commission, 2016). Distance in meters of road and hydrographic network, respectively with data from Instituto Geográfico Português (www.igeo.pt/) and SNIRH (http://snirh.apambiente.pt/) (SNIRH, 2016). Average annual temperature and annual temperature range in degrees multiplied by 10, and annual rainfall in mm gathered from BioClim (http://www.worldclim.org/bioclim) at 30 arc-second resolution (Hijmans *et al*., 2005). Primary productivity based on Normalized Difference Vegetation Index (NDVI) of each collected sample location used as a proxy of primary productivity (Pettorelli, 2013). The NDVI value for each record was calculated from satellite images supplied by MODIS (2016) (Moderate Resolution Imaging Spectroradiometer; http://modis.gsfc.nasa.gov) at a spatial resolution of 250m. Proxy of Egyptian mongoose, European rabbit and Red-legged partridge abundances (x animals/400ha) calculated through the number of animals hunted in each area and during the month, where and when each Egyptian mongoose sampled was collected (data from ICNF, *unpublished data*). Each variable was represented by mean

values of the 2 × 2 km grid cell, considering the vital area of the Egyptian mongoose (Palomares & Delibes, 1991*a*).

In light of current knowledge, this work is unprecedented, as no other research study on opportunistic carnivorous mammals produced or combined such a wide variety of biological data with physical components of the environment to unravel intrinsic ecological and demographic processes, from such a large sample. This knowledge is anticipated to be useful in the management of this species in the Portuguese territory and simultaneously in the understanding and monitoring of the impacts caused by global changes.

The expansion of Egyptian mongoose populations, that has been taking place in recent decades (Barros & Fonseca, 2011; Barros *et al*., 2015), enabled us to collect specimens around the Ria de Aveiro, in the shrubs, rice paddies and wetlands of Rio Mondego, and even in the agricultural valleys of Castro Daire, which would be unlikely 30 years ago. In fact, this study provides baseline data for future comparisons. This is a fundamental study because: firstly, the species is in an expansion phase (Barros & Fonseca, 2011), with evidence of still being exploring new distributions, such as the district of Aveiro (*personal observation*; Bandeira *et al*., 2013), and of its entry into a new biogeographic region; secondly, because there are no previous studies on biometric parameters or on reproductive patterns; thirdly, because the Portuguese territory is witnessing the human exodus from villages to cities, and with it, the abandonment of rural areas, increasing the suitable habitat for the species (Barros & Fonseca, 2011); Finally, all specimens of the present study were collected before the reintroduction of the Iberian lynx (*Lynx pardinus*) in Portugal in 2014, a competitor which can kill Egyptian mongooses to decrease competition for resources (Palomares *et al*., 1995; Ruiz-Olmo, 2012).

1.1. MAIN OBJECTIVES

Considering the unstoppable course of global changes and knowing that the Egyptian mongoose is expanding across the Portuguese territory, the population dynamics and expansion odds of the species are expected to be regulated and shaped by a combination of genetic and ecological characteristics, together with intrinsic demographic processes. This

work thus intended to enlighten the ecological structure of natural populations of mongoose in relation to its historic distribution and the new expansion areas and to expose how global mechanisms, such as natural environment factors and climate change, may affect the performance and distribution of plastic/opportunistic species, potentially assisting the management of wild populations in the future.

The identification of patterns in resource use by the Egyptian mongoose was also pursued as a fundamental step to unravel key ecological processes of this species. The following objectives were thus outlined:

- to analyze differences in biometrics and body mass in relation to life-history stage, dissecting variations with respect to gender and age structure, between Portuguese regions including historic distribution (south of Portugal) and expansion areas (northern Portugal), throughout the seasons, and seeking potential interactions with the type of habitat and land use, human pressure, climatic conditions, or ecological parameters. Given the different biotic and abiotic factors in the expansion and historic areas, we hypothesize that they will induce differences in biometric variables between these two populations of mongooses;

- to analyze and compare body condition and spleen weight (as a predictor of immune competence) with individual traits such as age structure and gender, across Portuguese regions including historic distribution (south of Portugal) and expansion areas (northern Portugal), throughout the seasons, relating them to the type of habitat and land use, human pressure, climatic conditions, or ecological parameters. Since ecological conditions of the Egyptian mongoose from south of the Tagus River (historic) are more favorable than those from north (expansion), we hypothesized there would be differences in spleen weight and body condition between both populations. We included both variables in our analysis to control for increases in spleen weight due to pathological conditions, which should generally be accompanied by a poor body condition score. Further, we expected geographic and seasonal differences, as well as gender dimorphism, to influence spleen weight and body condition;

- to characterize and compare the diet of two major subpopulations, in the historic and expansion distribution areas (Northern and Southern of Tagus River) in relation to life-history stage, studying the effects exerted by environmental factors, human pressure, or

ecological parameters; to ascertain if the Egyptian mongoose diet is essentially based on the consumption of small game species, and to gain a notion of the ecological footprint of the species. Due to the wide array of ecological differences between the historic and expansion areas, we hypothesized that these populations would have different diets. It is important to identify these differences due to their influence on the species potential to impact the ecosystems, either by consuming key prey species in the historic area, or by disrupting ecological balances in the expasion area. Secondly, we hypothesized there would be geographical, seasonal and gender differences in diet. Thirdly, we presume that animals with better condition and body size, greater spleen weight and living in areas with higher primary productivity will capture and consume larger and more energetic prey, while animals with divergent biological parameters will invest in the intake of less energetic prey, which require less effort in capture and manipulation. Finally and with the goal of clarifying the general doubts within the hunting community we aim to ascertain if game species are indeed the most commonly consumed prey by mongooses;

- to analyze the reproductive systems of both genders, identifying litter size or length of the fertile period and compare these reproductive indicators across regions of historic and expansion distribution, ascertaining the influence of environmental and human factors or ecological parameters, and establishing if there is an energetic cost associated with reproductive success. Here, we hypothesized that males and females exhibit variation in the weight of their reproductive organs throughout the year that are indicative of the periods in which they are active or inactive. Further, we predict that reproductive timings vary geographically, and are influenced by body condition, food resources and immune function. Thirdly, we hypothesized that there is a trade-off between resource allocation in reproduction and immune competence, and that these trade-offs are gender-specific.

This study used state of the art ecological methods tackling the ecological and biological events frame of a species in expansion in Portugal. The results gathered here might have applicability to inform decisions on species management, thus providing a critical model for science, society, hunting activity and the management of wildlife that face enduring global changes.

1.2. THESIS STRUCTURE

The thesis is structured into seven chapters. The first chapter is dedicated to the general framework of this study and to the presentation of the main objectives. Chapters 2 to 5 are presented in a scientific article format, concerning the main topics: biometrics, immune competence and body condition, diet, and reproduction, respectively. In the sixth chapter, a bullet style summarization of the main results and conclusions is presented, and in the seventh and final chapter some of the limitations and gains of this work are outlined, followed by future work perspectives.

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CHAPTER 2

BIOMETRICS

2.1. Geographic variation and sexual dimorphism in body size of the Egyptian mongoose, *Herpestes ichneumon* in the western limit of its European distribution

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Research paper

Geographic variation and sexual dimorphism in body size of the Egyptian mongoose, *Herpestes ichneumon* in the western limit of its European distribution

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2.2. ABSTRACT

The Egyptian mongoose (*Herpestes ichneumon*) is a carnivore game species in Portugal, whose fundamental ecology remains to be fully explored. For instance, the physical features of this species have not yet been studied through a continuous wide area, as an entire country. In this work, the averages of eight biometric parameters and body mass of both genders were determined for 559 free-ranging animals from four age classes that were established by dental growth.

Sexual dimorphism in body size of adult Egyptian mongoose is reported for the first time in the Portuguese territory, with males being larger than females. Differences across regions are also evidenced, with animals from south of Tagus River being larger than those from the north.

Comparison with similar data available from other mongoose populations of the Mediterranean basin suggests that adult specimens from Portugal are less heavy than animals from Doñana in Spain and Israel, while males appear to be larger in body length.

Statistically significant differences encountered among gender, age and region, suggest that sexual selection, prey availability, human constrains and different habitat use patterns regulate the body size of this species.

KEYWORDS

Herpestes ichneumon; Egyptian mongoose; Iberian Peninsula; body size; body weight; sexual dimorphism.

2.3. INTRODUCTION

The Egyptian mongoose, (*Herpestes ichneumon*, Linnaeus, 1758) (Mammalia, Carnivora, Herpestidae) (Corbet, 1984; Wozencraft, 2005), is the only representative species of the *Herpestidae* in the Iberian Peninsula (Delibes *et al*., 1984; Dobson, 1998; Cabral *et al*., 2005). In Portugal, it is expanding quickly from south to north and from inland to the coastal areas (Barros & Fonseca, 2011) due to transitions of land-use and climate changes over time (Barros *et al*., 2015). Similarly, expansion also seems to be occurring in Spain (Talegón & Parody, 2009; Recio & Virgós, 2010; Balmori & Carbonell, 2012). It is a small hunting species under the Portuguese law ("Decreto-Lei nº 201/2005") that is captured through density correction actions.

In Portugal, there is only one study focusing on Egyptian mongoose biometrics, being restricted to 38 adult specimens from Serra de Grândola, in the south, which reported that the species is not sexually dimorphic (Rosalino *et al*., 2005). Another study in Spain presented a significant difference in body mass between genders, but none in other metrics (Palomares, 1990). To our knowledge, there are no studies with mongooses across a wide region covering different ecological and climate amplitudes, which could elucidate if and how both body traits and sexual dimorphism change along with variations in environmental conditions.

Generalist and versatile carnivore species commonly have wide geographic distributions where the different populations are under contrasted climatic and environmental conditions, including large differences in the presence and abundance of prey species and competitors (Lucherini *et al*., 2006). Under such scenario, each population is subjected to diverse kinds of selection pressures, which can result in variations of body size and sexual dimorphism (eg. Ralls & Harvey, 1985; Thurber & Peterson, 1991; Lucherini *et al*., 2006; Yom-Tov *et al*., 2007*a*; Yom-Tov *et al*., 2007*b*; Virgós *et al*., 2011). Dissimilarities in biometric measurements may thus be related with differences in food availability and energy metabolism due to temperature variation or climatic change (Yom-Tov *et al*., 2006, Yom-Tov *et al*., 2008; Yom-Tov *et al*., 2010*a*; Yom-Tov *et al*., 2010*b*; Virgós *et al*., 2011). On the other hand, effects of anthropogenic habitat fragmentation and introduction of new competitors have been identified as drivers for body size variations (Sidorovich *et*

al., 1999; Lomolino & Perault, 2007). Because body size is a pivotal trait related to other life history traits and, ultimately, to fitness (Peters, 1983; Calder, 1984), studies dealing with geographic changes in body size are in the core of evolutionary ecology.

Sexual size dimorphism has usually been explained by niche separation and sexual selection (Ralls, 1976; Ralls & Harvey, 1985). Moreover, the body size of mammals is determined during the growth period (Yom-Tov *et al*., 2007*b*; Yom-Tov & Geffen, 2011) and depends on multiple factors, including the gestation length, reproductive rate, home range size, habitat productivity, metabolism, feeding niche or population densities (Thom *et al*., 2004; Webster *et al*., 2004; Lomolino & Perault, 2007). In Egyptian mongoose, Palomares (1993) demonstrated that body mass of males in Doñana (Spain) was the unique factor that explained the variation of its reproductive behavior, whereas home-range size was correlated with weight, negatively for females but positively for males, suggesting that different processes affect the relationship between size and home range area between genders: larger females tend to better defend resource areas, maintaining themselves in reduced areas, while larger males, being territorial, will have further access to females (Palomares, 1994). Because home range size, reproductive tactics and metabolism can also be influenced by factors with a clear geographic component, sexual size dimorphism is expected to vary across regions with different environmental conditions (Ralls & Harvey, 1985; Wigginton & Dobson, 1999).

In this context, the present work aims to provide new insights into the geographic variations of Egyptian mongoose biometrics, building on a robust and large-scale sampling approach that expands biometrics data largely beyond the temporal and spatial scales of the studies reported so far.

In particular, by covering all environments of the contemporaneous distributional range of the species in Europe (Barros & Fonseca, 2011; Barros *et al*., 2015), we aimed to describe and analyze how regional environmental features differences can shape age- and gender-related biometrics. We assume that the conditions from north of the Tagus River are distinctly less favorable to the ecology of the Egyptian mongoose compared to those of the south, since human pressure and habitat fragmentation are much higher in the north, while average temperatures, food availability and refuge seem more favorable in the south (see CIGeoE, 2016; INE, 2016; Instituto da Conservação da Natureza e das Florestas,

unpublished data). Since body size behaves as a barometer, rising and falling as the different forces exert their pressure (Yom-Tov & Geffen, 2011), geographic differences are expected to have influence on body size, presumably originating smaller individuals in the north and larger in the south.

2.4. MATERIAL AND METHODS

2.4.1. *SAMPLING PROCEDURES*

Sampling of free-ranging Egyptian mongooses was conducted between January 2008 and December 2014. All specimens were obtained from hunting activities and accidental road kills, in compliance with legal requirements and with permits from the competent authorities. Carcasses were labeled with the date and place of collection and stored at -20°C until the day of measurement and sample collection. Thawed carcasses were sexed, weighted, and measured. Measurements collected were snout-tail length (STL), tail length (TL, terminal hairs not included), head and body length (HBL), right hind leg length (RHLL), right hind foot length (RHFL), shoulder height (SH), neck perimeter (NP) and head width (HW), following the standard mammal measurement methods (eg. Palomares & Delibes, 1992; Castells & Mayo, 1993; Rosalino *et al*., 2005). In order to describe and compare different body weights and size parameters of males and females, within and across each age stage, the age of each specimen was determined by analysis of its dentition, following head removal and enzymatic cleaning for twelve hours using Neutrase 0.8L (provided by Univar, Maia, Portugal). Each specimen was assigned to one of four age classes: adults over one year of age, sub-adults between nine and twelve months, juveniles type II between five-and-a-half and nine months, and juveniles type I between two-and-a-half and five-and-a-half months of age. Skulls with completely developed definitive dentition were assigned to the adult class (Palomares & Delibes, 1992). For this purpose, all four canine teeth were checked to ascertain that all the definitive teeth were completely closed at their base (personal observations made by the same biologist to reduce operator variation). Skulls with 40 definitive teeth (Ben-Yaacov & Yom-Tov, 1983), but with some still growing, were assigned to the sub-adult age class (Palomares & Delibes, 1992). Active

tooth growth was determined based on large insertion spaces and at least one canine tooth presenting an open hole at its base (*personal observation*), meaning that the apical root foramen was still open (Grue & Jensen, 1979). Animals whose skulls presented all 40 teeth, but including at least one milk tooth, were assigned to the juvenile type II class (Palomares & Delibes, 1992). Finally, animals without molars and presenting only 32 teeth were assigned to the juvenile type I age class (Ben-Yaacov & Yom-Tov, 1983). No animals under the age of 2.5 months (cubs) were sampled, likely because mongooses do not emerge from their burrows before that period (Ben-Yaacov & Yom-Tov, 1983).

2.4.2. *STUDY AREA*

Wild Egyptian mongoose specimens were collected from 13 of 17 districts of continental Portugal where the species is distributed. The study area encompasses both the Atlantic and Mediterranean biogeographical regions (Costa *et al*., 1998; Rivas-Martínez *et al*., 2004). Depending on the origin of the harvest, each sample was assigned to a particular region, north or south of the Tagus River, which may be considered a geographical barrier for the species, bearing in mind that, until the 90s (of the XX century), the distribution of the Egyptian mongoose was more concentrated in the south of the Tagus River (Borralho *et al*., 1996; Barros & Fonseca, 2011). Regarding land cover and habitat characteristics of each region, flora found in the north is mainly characterized by monoculture of *Eucaliptus* sp., which replaced a large part of the areas occupied by *Pinus pinaster*, *Quercus robur*, *Salix* sp. or *Alnus glutinosa* (Alves *et al*., 2009). In the south, the prevailing flora consists of *Quercus suber*, with well-populated *Olea europaea* and *Quercus ilex* areas (Alves *et al*., 2009). In contrast with the south, the north is also characterized by higher human density, more kilometers of road, much more fragmented habitats and also more altered by human action, greater number of ridges with higher altitude, more rocky areas, denser hydrographic network, lower temperatures, higher rainfall (see CIGeoE, 2016; INE, 2016) and lower densities of prey target species as rabbits, rodents, red-legged partridges or reptiles (Instituto da Conservação da Natureza e das Florestas, *unpublished data*; Loureiro *et al*., 2008).

To complement study area information and to give insights into the biogeographic differences that each population faces in the north and south of Tagus River, we provide

bioclimatic and environmental data as supplementary figures (Figures A1.1 to A1.4) [number of hectares of each habitat type (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests and agriculture areas) were retrieved from Corine Land Cover (2006) with spatial resolution of 250m. Mean altimetry value (data SRTM, NASA, resolution of 30 meters) was gathered using the ASTER Global Digital Elevation Model platform (http://gdem.ersdac.jspacesystems.or.jp/) (ASTER, 2016). Number of inhabitants per km^2 in each grid was investigated using data from Eurostat per kilometer (http://epp.eurostat.ec.europa.eu/) (European Commission, 2016), distance in meters of road and hydrographic network, respectively with data from Instituto Geográfico Português (www.igeo.pt/) and SNIRH (http://snirh.apambiente.pt/) (SNIRH, 2016). Average annual temperature in degrees multiplied by 10 and annual rainfall in mm were gathered from BioClim (http://www.worldclim.org/bioclim) at 30 arc-second resolution (Hijmans *et al*., 2005). Finally, primary productivity based on Normalized Difference Vegetation Index (NDVI) of each collected sample location was used as a proxy of primary productivity (Pettorelli, 2013). The NDVI value for each record was calculated from satellite images supplied by MODIS (2016) (Moderate Resolution Imaging Spectroradiometer; http://modis.gsfc.nasa.gov) at a spatial resolution of 250m. Each variable was represented by mean values of the 2 x 2 km grid cell, considering the home-range of the Egyptian mongoose (Palomares & Delibes, 1991)].

2.4.3. *STATISTICAL PROCEDURES*

All weight and biometric variables were tested for normality with Kolmogorov-Smirnov Test (with correction of Lilliefors for the significance level) (Zar, 1999) and with Shapiro-Wilk W Test (eg. Zuur *et al*., 2009).

In order to combine the information regarding weight and six of the physical measures mentioned up above (excluding tail length and head and body length, to avoid repeating snout-tail length), all seven variables were transformed into a single factor, named body size, for each specimen. This factor was extracted using a Principal Component Analysis (PCA), which yielded a single variable that represents the estimated body size, based on

the covariance matrix of various measures. Variables were retained in the new PCA factor when loadings were higher than 0.70.

The effects of gender, age and region (north and south of Tagus river) (independent variables) on size components retained by PCA (PCA1, dependent variable) were analyzed by means of Generalized Linear Mixed Models (GLMM), using the district as a random factor to control for non-independence of samples from the same geographic area. Only *p* values lower than 0.05 were considered statistically significant. Model selection was performed using the Akaike information criterion (AIC) following Zuur *et al*. (2009) procedure. In brief, we ranked all possible models using AICc (Burnham & Anderson, 2002) and only models with differences lower than 2 in values of AICc of the i^{th} model and the minimum AICc value ($\Delta AICc<2$) were considered as explanatory (Johnson & Omland, 2004). If several models were selected, an averaging modeling approach was used (Burnham & Anderson, 2002). Mean and standard deviation parameters were determined for each biometric variable of each age class and gender using one way ANOVA test and applying the Bonferroni correction for alpha (McDonald, 2014).

All statistical analyses were performed using R software (version 2.13.2) with the *lme4* (Bates *et al*., 2014) and *MuMIn* (Barton & Barton, 2015) packages.

2.5. RESULTS

The sampling strategy yielded 678 Egyptian mongooses, but only 559 animals, whose skulls were processed and allowed appropriate age classification, were considered for the present study. Pregnant females were discarded to avoid interference in weight measurements. In total, 292 females and 267 males of Egyptian mongoose were considered, of which 154 were from north and 405 from south of Tagus River (Figure 2.1, Table 2.1); 22 were from Atlantic and 537 from Mediterranean biogeographic region. Regarding specimen distribution by age class, 298 were adults, 91 were sub-adults, 98 were type II juveniles and 72 were type I juveniles (Table 2.1).

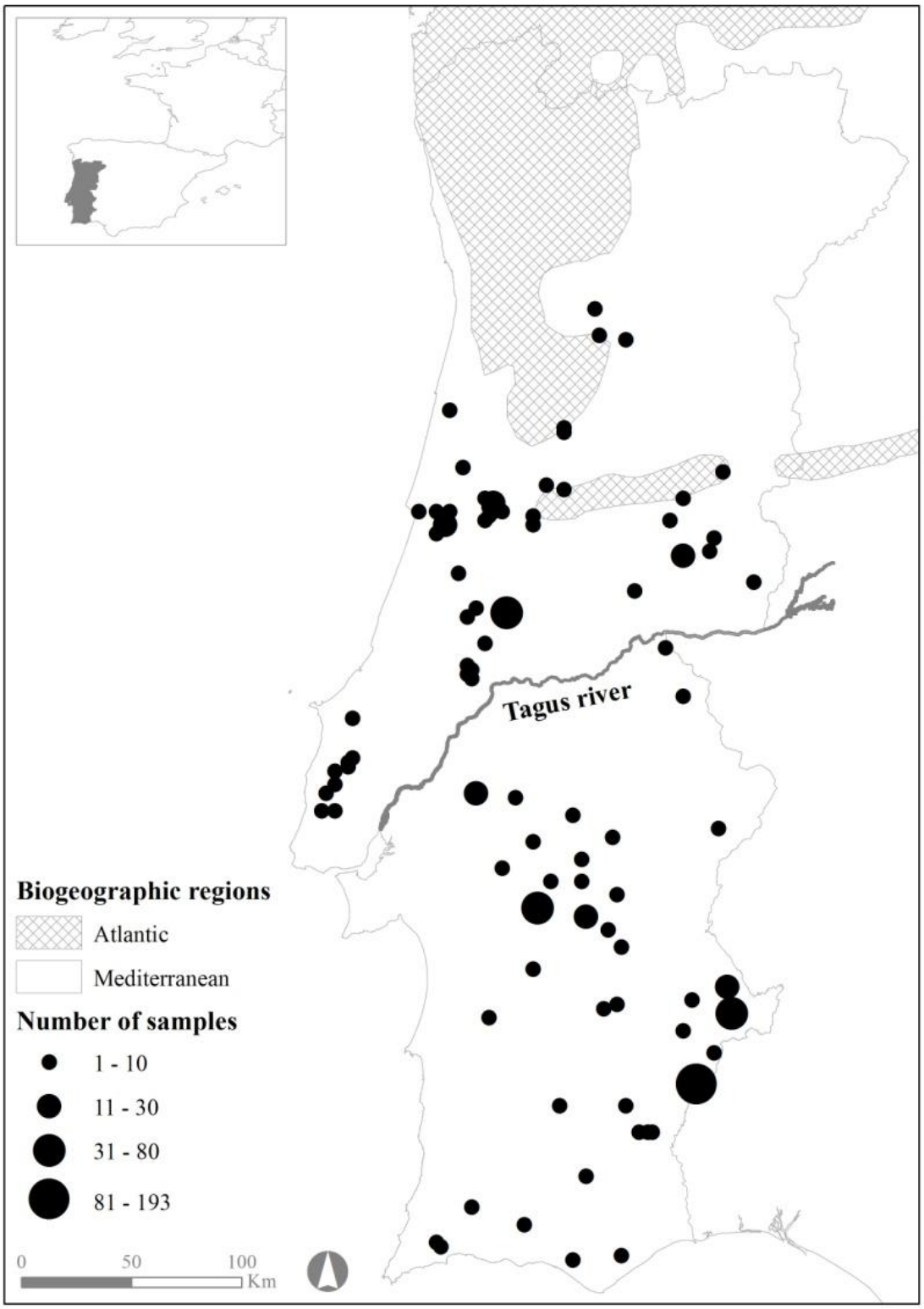

Figure 2.1. Locations and number of samples of the Egyptian mongoose specimens under study.

Table 2.1. Number of Egyptian mongoose samples obtained in each study region (north and south of Tagus River), by age class and gender.

Age Class	Gender	Region	
		North	**South**
Adult	**Female**	52	114
	Male	41	91
Sub-adult	**Female**	14	33
	Male	17	27
Juvenile II	**Female**	6	35
	Male	10	47
Juvenile I	**Female**	4	34
	Male	10	24
Total	**Female**	76	216
	Male	78	189

Biometric analyses revealed significant differences between genders in all biometric measures (with exception of tail length and shoulder height) for adults, with males being 13% heavier and 3% longer than females. Seven biometric measurements of adult males exceeded those of females (Table 2.2).

Table 2.2. Biometrics of adult Egyptian mongoose (BW – Body Weight; STL – Snout-Tail Length; TL – Tail Length; HBL – Head and Body Length; RHLL – Right Hind Leg Length; RHFL – Right Hind Foot Length; SH – Shoulder Height; NP – Neck Perimeter; HW – Head Width), whose values are given as mean ± standard deviation (top), range (bottom). n is the sample size; F the statistic value and *p* the significance value of one way ANOVA test. (*p* significant values at the 0.0056 level with a Bonferroni adjustment for alpha are in bold).

Adult	**BW (g)**	**STL (cm)**	**TL (cm)**	**HBL (cm)**	**RHLL (cm)**	**RHFL (cm)**	**SH (cm)**	**NP (cm)**	**HW (cm)**
Females	2145.34 ± 342.74 (1142-2957)	95.50 ± 5.35 (79 – 106.2)	43.24 ± 2.77 (35 – 50.6)	52.27 ± 3.81 (40 - 61)	22.44 ± 1.37 (17.6 – 25.5)	9.30 ± 0.42 (8.2 – 10.4)	17.98 ± 2.45 (12.5 – 27.2)	19.54 ± 1.53 (14 - 24.9)	8.57 ± 0.65 (6.6 - 10)
Males	2417.34 ± 444.04 (1200 - 3694)	98.53 ± 5.83 (76 – 110.3)	43.95 ± 3.39 (25.5 – 51.5)	54.59 ± 4.14 (41 – 63.3)	23.27 ± 1.35 (18.3 – 26.2)	9.59 ± 0.53 (8.1 - 11)	18.61 ± 2.18 (13.5 – 26.2)	20.36 ± 1.65 (16.2 – 24.5)	8.97 ± 0.70 (7.4 - 11)
Total	2265.82 ± 412.97 (1142 - 3694)	96.85 ± 5.76 (76 – 110.3)	43.55 ± 3.07 (25.5 – 51.5)	53.29 ± 4.12 (40 – 63.3)	22.81 ± 1.42 (17.6 – 26.2)	9.43 ± 0.49 (8.1 - 11)	18.26 ± 2.35 (12.5 – 27.2)	19.91 ± 1.64 (14 – 24.9)	8.74 ± 0.70 (6.6 - 11)
n	F=166 M=132 T=298	F=166 M=132 T=298	F=166 M=132 T=298	F=166 M=132 T=298	F=159 M=129 T=288	F=157 M=130 T=287	F=166 M=132 T=298	F=165 M=132 T=297	F=163 M=130 T=293
F	35.62	21.77	3.98	25.17	26.81	27.9	5.42	19.67	25.50
p	**< 0.00001**	**0.000005**	0.046979	**0.000001**	**< 0.00001**	**< 0.000001**	0.020549	**0.000013**	**0.000001**

For sub-adults, only a significant difference in tail length was found, with females presenting longer tails than males (Table 2.3). However, sub-adult females presented a tendency to be heavier and to have longer total body length, as well as longer hind leg, neck perimeter and head width (Table 2.3).

Table 2.3. Biometrics of sub-adult Egyptian mongoose (BW – Body Weight; STL – Snout-Tail Length; TL – Tail Length; HBL – Head and Body Length; RHLL – Right Hind Leg Length; RHFL – Right Hind Foot Length; SH – Shoulder Height; NP – Neck Perimeter; HW – Head Width), whose values are given as mean ± standard deviation (top), range (bottom). n is the sample size; F the statistic value and *p* the significance value of one way ANOVA test. (*p* significant values at the 0.0056 level with a Bonferroni adjustment for alpha are in bold).

Sub-adult	**BW (g)**	**STL (cm)**	**TL (cm)**	**HBL (cm)**	**RHLL (cm)**	**RHFL (cm)**	**SH (cm)**	**NP (cm)**	**HW (cm)**
Females	2025.17 ± 341.69 (1151 - 2813)	93.19 ± 4.70 (83.2 - 105.5)	43.49 ± 2.36 (38 – 50.6)	49.69 ± 3.55 (42.8 – 57.4)	21.91 ± 1.40 (17 –25.2)	9.16 ± 0.39 (8.3 - 9.9)	17.17 ± 1.79 (13.2 - 23.1)	19.26 ± 1.89 (14.3 - 24.6)	8.41 ± 0.61 (6.5 - 10)
Males	1896.57 ± 377.94 (1189 - 2770)	91.00 ± 5.78 (76 - 102.7)	41.38 ± 3.55 (34.2 – 50.7)	49.62 ± 3.92 (40.4 - 59)	21.59 ± 1.73 (20–24.6)	9.28 ± 0.51 (8.1 - 10.2)	18.01 ± 3.36 (12.6 - 26)	18.68 ± 1.59 (14.2 - 21.5)	8.28 ± 0.71 (6.7 - 10)
Total	1962.99 ± 363.45 (1151 - 2813)	92.13 ± 5.34 (76 – 105.5)	42.47 ± 3.16 (34.2 – 50.7)	49.66 ± 3.71 (40.4 - 59)	21.76 ± 1.56 (17 – 25.2)	9.22 ± 0.45 (8.1 – 10.2)	17.58 ± 2.68 (12.6 - 26)	18.98 ± 1.77 (14.2 – 24.6)	8.34 ± 0.66 (6.5 - 10)
n	F=47 M=44 T=91	F=47 M=44 T=91	F=47 M=44 T=91	F=47 M=44 T=91	F=47 M=44 T=91	F=47 M=42 T=89	F=47 M=44 T=91	F=47 M=44 T=91	F=47 M=44 T=91
F	2.906	3.93	11.30	0.01	0.98	1.56	2.263	2.54	0.93
p	0.091766	0.050525	**0.001142**	0.925786	0.325803	0.214312	0.136035	0.114349	0.338178

In juveniles (types II and I), no variation was noted between genders (Tables 2.4 and 2.5). With the exception of head width, biometrics for male type II juveniles exhibited a slight tendency to be higher than females (Table 2.4); in contrast, biometrics of type I juveniles were very similar between genders (Table 2.5).

Table 2.4. Biometrics of juvenile type II Egyptian mongoose (BW – Body Weight; STL – Snout-Tail Length; TL – Tail Length; HBL –Head and Body Length; RHLL – Right Hind Leg Length; RHFL – Right Hind Foot Length; SH – Shoulder Height; NP – Neck Perimeter; HW – Head Width), whose values are given as mean ± standard deviation (top), range (bottom). n is the sample size; F the statistic value and *p* the significance value of one way ANOVA test. (*p* significant values at the 0.0056 level with a Bonferroni adjustment for alpha are in bold).

Juvenile II	**BW (g)**	**STL (cm)**	**TL (cm)**	**HBL (cm)**	**RHLL (cm)**	**RHFL (cm)**	**SH (cm)**	**NP (cm)**	**HW (cm)**
Females	1559.61 ± 356.67	85.93 ± 6.27	40.28 ± 3.73	45.65 ± 3.84	20.71 ± 1.74	9.02 ± 0.60	15.53 ± 1.96	17.12 ± 1.75	8.10 ± 0.58
	(723 - 2417)	(72.1 - 99)	(32.2 – 47.6)	(36.3 – 53.7)	(17.7 – 24.2)	(6.7 – 10.3)	(12.2 - 21)	(13.2 – 20.4)	(7.1 - 10)
Males	1623.00 ± 436.94	87.70 ± 7.50	40.80 ± 4.06	46.90 ± 4.28	21.23 ± 1.44	9.21 ± 0.53	16.41 ± 2.77	17.46 ± 1.90	8.01 ± 0.65
	(627 - 3085)	(65.5 - 101.6)	(26.8 - 47)	(35.4 – 58.4)	(17.6 – 23.8)	(8 - 10)	(12.2 - 25)	(12 - 22)	(6.5 – 9.5)
Total	1596.48 ± 404.56	86.96 ± 7.04	40.59 ± 3.91	46.37 ± 4.13	21.01 ± 1.59	9.13 ± 0.57	16.04 ± 2.49	17.31 ± 1.84	8.05 ± 0.62
	(627 - 3085)	(65.5 - 101.6)	(26.8 – 47.6)	(35.4 – 58.4)	(17.6 – 24.2)	(6.7 – 10.3)	(12.2 - 25)	(12 - 22)	(6.5 - 10)
n	F=41	F=41	F=41	F=41	F=41	F=40	F=41	F=41	F=41
	M=57	M=57	M=57	M=57	M=57	M=57	M=57	M=57	M=57
	T=98	T=98	T=98	T=98	T=98	T=97	T=98	T=98	T=98
F	0.583	1.52	0.42	2.22	2.67	2.71	3.034	0.813	0.46
p	0.447021	0.220319	0.518524	0.139255	0.105323	0.102862	0.084746	0.369570	0.497109

Table 2.5. Biometrics of juvenile type I Egyptian mongoose (BW – Body Weight; STL – Snout-Tail Length; TL – Tail Length; HBL – Head and Body Length; RHLL – Right Hind Leg Length; RHFL – Right Hind Foot Length; SH – Shoulder Height; NP – Neck Perimeter; HW – Head Width), whose values are given as mean ± standard deviation (top), range (bottom). n is the sample size; F the statistic value and *p* the significance value of one way ANOVA test. (*p* significant values at the 0.0056 level with a Bonferroni adjustment for alpha are in bold).

Juvenile I	**BW (g)**	**STL (cm)**	**TL (cm)**	**HBL (cm)**	**RHLL (cm)**	**RHFL (cm)**	**SH (cm)**	**NP (cm)**	**HW (cm)**
Females	940.21 ± 346.40 (320 -1744)	70.42 ± 10.82 (43.5 - 89.1)	32.12 ± 5.54 (19.5 – 40.5)	38.30 ± 5.71 (24 – 50.9)	17.83 ± 1.85 (13.1 – 20.1)	7.98 ± 0.85 (5.9 - 9.1)	12.99 ± 1.84 (9.9 - 16)	14.53 ± 2.26 (9 - 19)	7.31 ± 0.60 (6.1 - 8.8)
Males	938.59 ± 341.01 (394 - 2147)	71.17 ± 10.34 (52.2 - 103.8)	31.20 ± 5.34 (20.5 – 47.2)	39.97 ± 5.47 (30.5 – 56.6)	18.11 ± 1.84 (12.7 – 23.1)	8.05 ± 0.72 (5.9 - 9.6)	13.74 ± 2.30 (10.5 - 18.5)	14.50 ± 1.97 (10.5 - 21)	7.25 ± 0.67 (6.3 - 9.5)
Total	939.44 ± 341.44 (320 - 2147)	70.77 ± 10.53 (43.5 - 103.8)	31.69 ± 5.43 (19.5 – 47.2)	39.09 ± 5.62 (24 – 56.6)	17.96 ± 1.84 (12.7 – 23.1)	8.01 ± 0.79 (5.9 – 9.6)	13.35 ± 2.09 (9.9 – 18.5)	14.52 ± 2.12 (9 - 21)	7.28 ± 0.63 (6.1 – 9.5)
n	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72	F=38 M=34 T=72
F	0.0004	0.089	0.514	1.593	0.412	0.155	2.347	0.005	0.152
p	0.984112	0.766326	0.475978	0.211097	0.523196	0.695178	0.130048	0.941322	0.698059

The proportion of variance explained by the factor body size extracted from PCA clumped from all seven variables was 73.5%, with an eigenvalue of 5.145 [loadings for each variable are provided as supplementary material (Table A1.1)]. The factor body size (PCA1) was analyzed against the independent variables region, gender and age, by means of GLMM. The district was used as a random factor as it exerted statistically significant effects on gender (p<0.0290), age (p<0.0001), region*age (p<0.0012) and gender*age (p<0.0100) (Table 2.6).

Table 2.6. Results obtained from GLMM analyses to examine gender, age and/or region variables as categorical predictors of body size (explained as PCA1 retained by PCA analysis of six measured biometrics and weight) of Egyptian mongoose. District was used as random factor to control for spatial autocorrelation of samples. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold).

Effect	df	MS	F	*p*
Intercept	1	9.261	22.624	**<0.0001**
DISTRICT	12	0.890	2.971	**0.0005**
REGION	1	0.029	0.098	0.7542
GENDER	1	1.436	4.795	**0.0290**
AGE	3	58.285	194.598	**<0.0001**
REGION x GENDER	1	<0.001	<0.001	1.0000
REGION x AGE	3	1.602	5.348	**0.0012**
GENDER x AGE	3	1.145	3.822	**0.0100**
REGION x GENDER x AGE	3	0.278	0.929	0.4266
Error	512	0.300		

After ranking all possible models using AICc, based on all possible combinations of independent variables and their interaction, only one model with ΔAICc lower than 2 was obtained. The model considered as explanatory presented the following variables and interactions: age + region + gender + age x region + age x gender, with an AICc of 940.4 (df=539) (Tables 2.7 and 2.8).

Table 2.7. Model selection, using AICc, for the effect of age, region, gender and their interactions on body size (PCA1 of six measured biometrics and weight) of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	df	AICc	ΔAICc	AICc weight
AGE + REGION + GENDER + AGE x REGION + AGE x GENDER	14	941.18	0.00	0.61
AGE + GENDER + AGE x GENDER	10	943.51	2.34	0.19
AGE + REGION + GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	15	944.11	2.94	0.14
AGE + REGION + GENDER + AGE x GENDER	11	948.36	7.19	0.02
AGE + GENDER	7	948.69	7.51	0.01
AGE + REGION + GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER + AGE x REGION x GENDER	18	948.79	7.62	0.01
AGE + REGION + GENDER + AGE x REGION	11	949.52	8.34	0.01
AGE + REGION + GENDER + AGE x GENDER + REGION x GENDER	12	952.71	11.53	0.00
AGE + REGION + GENDER + AGE x REGION + REGION x GENDER	12	952.89	11.71	0.00
AGE + REGION + GENDER	8	953.55	12.37	0.00
AGE + REGION + GENDER + REGION x GENDER	9	957.91	16.73	0.00
AGE + REGION + AGE x REGION	10	972.84	31.67	0.00
AGE	6	974.54	33.37	0.00
AGE + REGION	7	979.44	38.26	0.00

Table 2.8. Effects of the model considered as explanatory for body size (PCA1 of six measured biometrics and weight) of the Egyptian mongoose in Portugal.

Variables		**Estimate**	**Std. Error**	**t value**
Intercept		0.215	0.087	2.486
GENDER	Male	0.463	0.066	7.034
AGE	Juvenile 1	-1.633	0.188	-8.679
	Juvenile 2	-0.594	0.177	-3.351
	Sub-adult	-0.190	0.136	-1.404
REGION	South	0.110	0.105	1.041
AGE x REGION	Juvenile 1 x South	-0.717	0.184	-3.904
	Juvenile 2 x South	-0.422	0.173	-2.434
	Sub-adult x South	-0.068	0.143	-0.477
AGE x GENDER	Juvenile 1 x Male	-0.485	0.149	-3.253
	Juvenile 2 x Male	-0.254	0.131	-1.945
	Sub-adult x Male	-0.521	0.134	-3.874

Results obtained from this model demonstrated that males were larger than females (Table 2.8). Animals from south of the Tagus River were larger in body size than those from north of the river (Table 2.8). Adults from the South were larger than other animal classes of the same region, and adult males were larger than males from other age classes (Table 2.8). For details on each factor, refer to means presented in Tables 2.2 to 2.5 and to graphics presented in Figures 2.2 and 2.3.

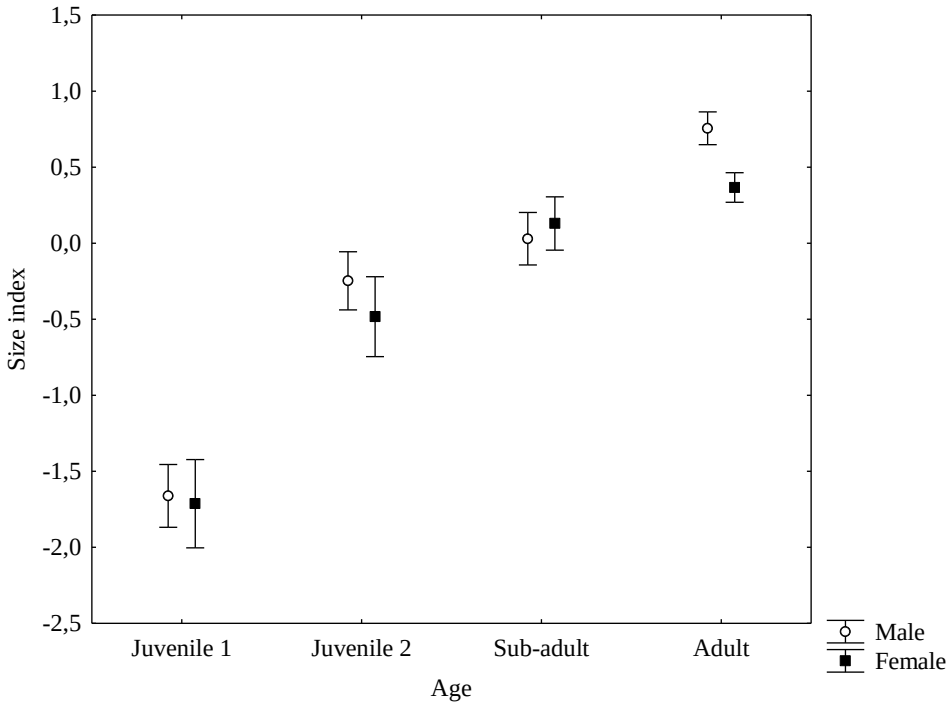

Figure 2.2. Means of size index calculated by PCA using weight and six physical measures of the Egyptian mongoose observed for each gender across the age class. Vertical bars denote 95% confidence intervals.

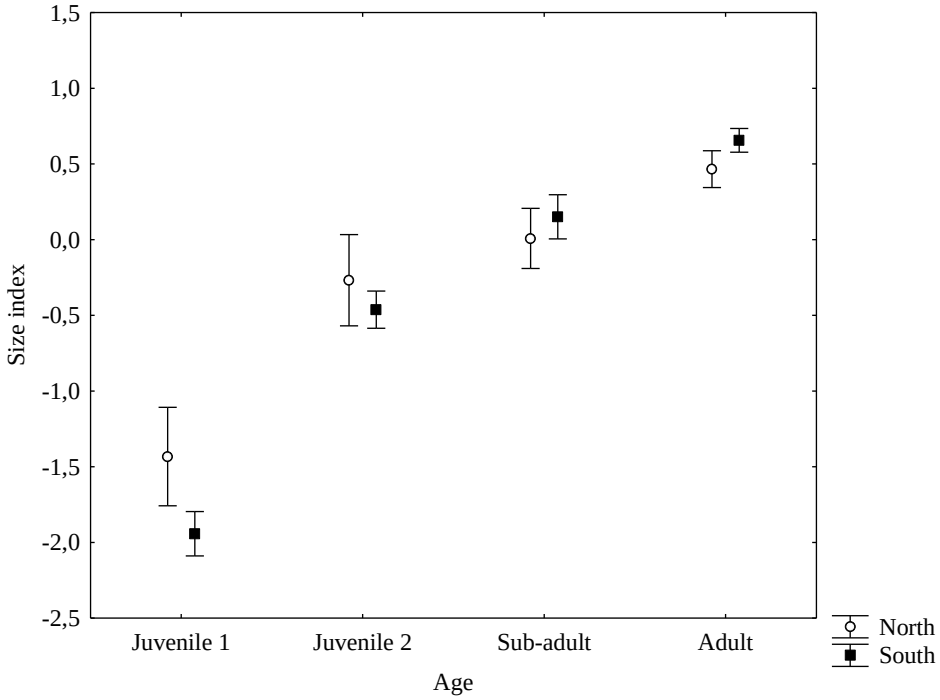

Figure 2.3. Means of size index calculated by PCA using weight and six physical measures of the Egyptian mongoose observed for each region across the age class. Vertical bars denote 95% confidence intervals.

The comparison of our biometric measurements in mainland Portugal with data from two other Mediterranean regions shows that body weights (BW) of adult females from this sample were 31% and 23% lower than those from Doñana (Spain) and Israel, respectively (Table 2.9). The same trend is observed in males, who are 30% and 22% lighter than those from Doñana and Israel (Table 2.9). Adult males in Portugal are on average 1% and 6% longer than males from Doñana and Israel (Table 2.9). Adult females from Doñana population display a total length (STL) 1% and 5% longer than females from Portugal and Israel, respectively (Table 2.9).

Table 2.9. Biometrics of adult Egyptian mongoose (BW – Body Weight; STL – Snout-Tail Length; TL – Tail Length; HBL – Head and Body Length; RHLL – Right Hind Leg Length; RHFL – Right Hind Foot Length; SH – Shoulder Height; NP – Neck Perimeter; HW – Head Width), whose values are given as mean for both genders. Each region is related with a study: a) Portugal – this study; b) Grândola (Portugal) – Rosalino *et al*., 2005; c) Doñana (Spain) – Palomares & Delibes, 1992; d) Israel – Ben-Yaacov & Yom-Tov, 1983. (The values from this study are presented in bold; n is the sample size.)

Gender	**Female**				**Male**			
Regions	a) **Portugal**	b) **Grândola (Portugal)**	c) **Doñana (Spain)**	d) **Israel**	a) **Portugal**	b) **Grândola (Portugal)**	c) **Doñana (Spain)**	d) **Israel**
BW (g)	**2145.34**	1946	2823.3	2640	**2417.34**	2013	3142.2	2953
STL (cm)	**95.50**	89.64	96.30	91.50	**98.53**	88.77	97.63	93.20
TL (cm)	**43.24**	39.24	43.33	42.50	**43.95**	39.71	44.96	39.70
HBL (cm)	**52.27**	50.40	52.97	49.00	**54.59**	49.06	52.67	53.50
RHLL (cm)	**22.44**	19.07	21.08	-	**23.27**	19.08	21.29	-
RHFL (cm)	**9.30**	8.32	9.06	9	**9.59**	8.43	8.69	10
SH (cm)	**17.98**	-	-	-	**18.61**	-	-	-
NP (cm)	**19.54**	17.41	-	-	**20.36**	18.52	-	-
HW (cm)	**8.57**	-	-	-	**8.97**	-	-	-
n	**166**	22	12	5	**132**	16	9	12

2.6. DISCUSSION

The analysis of a high number of geographically widespread Egyptian mongoose specimens evidenced sexual dimorphism in body size of adult males and females, the first being larger and heavier, similar to what is reported for other species of sympatric carnivores (Petrov *et al*., 1992; Beltrán & Delibes, 1993; Ruiz-Olmo, 1995; Palomo & Gisbert, 2002; Rosalino *et al*., 2005; Rodriguez-Refojos *et al*., 2011; Simón *et al*., 2012). This finding contrasts with previous research conducted on a regional scale in mainland Portugal that was limited to the population of Serra de Grândola, in the littoral south, with 38 studied samples for which no significant differences between genders in body size were apparent (Rosalino *et al*., 2005). In a more recent review, it was acknowledged that males may be slightly larger than females, but without evident morphological differences (Rosalino & Chambel, 2012). However, during the '90s, 21 specimens from the population of Doñana (Spain) were studied and significant differences in body mass of both genders were reported, with males being heavier than females, but with no differences in body length (Palomares, 1990; Palomares & Delibes, 1992). In Israel, with a sampling of 17 specimens, males were significantly heavier and larger than females (Ben-Yaacov & Yom-Tov, 1983). The contrasting results concerning the population of Serra de Grândola (Rosalino *et al*., 2005) could be due to two non-mutually exclusive reasons, the first of which is the reduced sample size. However, it should be emphasized that in the Israeli population, gender differences were still registered despite limited sampling (Ben-Yaacov & Yom-Tov, 1983). The second explanation could be due to unclearly defined age classes and inclusion of juveniles or sub-adults in the adult sampling group of Serra de Grândola (Rosalino *et al*., 2005). The lack of dental analysis in the latter study may have impaired definitive categorization of a specimen as an adult. In our sample, some juveniles presented weights and body sizes comparable to mature individuals, while some adults were smaller than juveniles, and dental analysis was indeed useful to resolve these issues. Results based on external characteristics alone should thus be interpreted with caution.

It is expectable that males increase their body size to compete with others, enabling access to females due to sexual selection (Lucherini *et al*., 2006; Lindenfors *et al*., 2007). The Egyptian mongoose male has been classified as following a polygyny mating tactic (Palomares, 1993). In such a system, a male with a larger body size has advantage in the

competition between males for mates, favoring sexual selection (King, 1989; Fairbairn, 1997). This selection will promote larger sizes that enhance the capability of males to dominate in contest, such as a fast growth rate and a large body size (Isaac, 2005). Sexual size dimorphism also can be important to promote different habitat use patterns for each gender, as registered in *Genetta genetta* (Rodriguez-Refojos *et al*., 2011) or *Martes foina* (Santos & Santos-Reis, 2010). Furthermore, and similar to what has been observed in *Mustela vison* (Thom *et al*., 2004), mongoose males may be larger than females in order to specialize in larger prey and, therefore, size could be functionally linked to killing, handling and feeding behavior. A larger neck circumference is also indicative of more powerful neck musculature (Radinsky, 1981*a*; 1981*b*) and male mongooses have it, providing them more strength to kill and handle preys. These differences reduce inter-sexual competition by exploiting different prey sizes (Lucherini *et al*., 2006). In Egyptian mongoose, male's and female's diets are somewhat dissimilar, males focusing more on mammals and presenting better body condition, while females apparently prefer reptiles (Rosalino *et al*., 2009; Bandeira *et al*., *unpublished data*). This difference may be linked to the fact that males consume more energetic prey because they need more resources to defend and patrol their territory, while females outweigh the reproduction costs consuming prey that require less energy and time to capture, such as reptiles (Rosalino *et al*., 2009).

The analyses of interactions between region and age show interesting geographic variations in body size. As adults, the Egyptian mongoose from south are largest, perhaps due to better food availability/quality, especially during the first months of life, where animals in optimal habitats become larger because they receive more food as they grow (eg. Rosatte *et al*., 1991; Thom *et al*., 2004; Yom-Tov *et al*., 2007*a*). In Portugal, the composition and the prey species richness differs geographically (eg. Mathias *et al*., 1999; Cabral *et al*., 2005; Loureiro *et al*., 2008). Game reserves, predominantly located in the south of Portugal, provide large concentrations of high quality prey, as is the case of European rabbits (Beja *et al*., 2008), the most energetic prey for mongoose (Delibes *et al*., 1984), that is scarcer in the north of the mainland. In addition, there is less human pressure, namely less people, anthropogenic habitat fragmentation and road network in this region comparatively to the north. In the case of *Meles meles*, it has been suggested that food availability/quality is the main driving force to maintain geographic differences in the body size (Virgós *et al*., 2011). Also in a study with *Vulpes vulpes*, it has been suggested that the

body size could vary due to changes of habitat, even over short distances (microgeographical), where the habitats with better quality favor larger and heavier foxes (Gortázar *et al*., 2000). Our results regarding mongooses seem to support these hypotheses, although more studies about the diet and habitat of both regions are needed to clarify these patterns.

Comparing our results with other Egyptian mongoose populations of the Mediterranean basin (except Grândola population it is within the area of our sample), it was found that the adult males and females of the Portuguese population are lighter than the population of Doñana (Spain) and Israel. However, in terms of physical biometrics, Portuguese males are larger than the counterparts, and Portuguese females are just smaller than Doñana. Presumably, there are various forces acting differently on each population (Ralls & Harvey, 1985; Thurber & Peterson, 1991; Lucherini *et al*., 2006), and possibly these differences may be related to pressures of, once again, food quality and availability (Yom-Tov *et al*., 2007*a*; Yom-Tov *et al*., 2008; McNab, 2010; Yom-Tov *et al*., 2010*b*; Rodriguez-Refojos *et al*., 2011), global warming (Yom-Tov *et al*., 2006; Yom-Tov *et al*., 2008; Yom-Tov *et al*., 2010*a*; 2010*b*), habitat fragmentation (Lomolino & Perault, 2007), habitat change (Gortázar *et al*., 2000), primary production (McNab, 2010) or competition with different sympatric species (Sidorovich *et al*., 1999; McNab, 2010). In a comparative study of three distinct populations of *Genetta genetta* in the Iberian Peninsula, a different preference for microhabitat associated to sexual dimorphism, along with temperature and prey availability, are suggested as factors that promote gender differences (Rodriguez-Refojos *et al*., 2011). In another study focused on *Vulpes vulpes* in Iberian Peninsula, it was found that increase of available food increases size (Yom-Tov *et al*., 2007*a*). On the other hand, competition with different species of carnivores vary from region to region, with consequences on the exposure and response to direct aggressions and menaces posed by adversaries of the same or unequal size (Sidorovich *et al*., 1999).

Beyond the sexual dimorphism reported, our initial predictions were confirmed in this study, as the specimens from south were larger than northern, and these differences could be broadly related to a multitude of environmental, ecological and climatic features. Moreover, an interesting future approach to consolidate these hypotheses could be built on

the trends emphasized by this study, comparing body size and body condition changes with trophic ecology of each gender across the whole distribution range of the species.

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CHAPTER 3

IMMUNE COMPETENCE
AND
BODY CONDITION

3.1. Life-history traits and seasonal effects drive spleen weight and body condition of a medium-sized carnivore, the Egyptian mongoose (*Herpestes ichneumon*)

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3.2. ABSTRACT

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a medium-sized carnivore that experienced a remarkable geographic expansion over the last three decades in the Iberian Peninsula, due to transitions of land-use and climate changes. In this study, we investigated the influence of species-related factors and abiotic effects on species performance and on spleen weight as a proxy for immunocompetence. For that purpose, body condition and spleen weight were determined for 518 animals gathered from hunting activities and road kills and related with body fat, as well as with life-history and environmental parameters.

Our results indicate that mongoose males have heavier spleens and distinctively suggest that spleen weight follows a seasonal variation, which may be related with the reproductive behavior and health status of individuals. Because the immune system is energetically costly, we expected spleen weight to be dependent on body condition, for which we confirmed a positive correlation. Consistently, body condition was also higher in males and in adult mongooses, which may be the result of forces shaping the trade-off between animal growth and reproduction.

Behavioural and physiological differences that intersect reproductive ecology and diet features, as well as season, seem to exert pronounced effects on life-history traits and spleen function and, possibly, on immunocompetence of Egyptian mongoose.

KEYWORDS

Herpestes ichneumon; carnivore; mongoose; Iberian Peninsula; spleen weight; body condition; immune competence.

3.3. INTRODUCTION

In Europe, the Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is restricted to the westernmost region of the Iberian Peninsula (Delibes *et al*., 1984; Dobson, 1998). In Portugal, its distribution range has been increasing northwards (Barros & Fonseca, 2011). This species is liable to be hunted under the Portuguese law ("Decreto-Lei nº202/2004" with the new wording given by "Decreto-Lei nº 201/2005") and in legal actions to correct its densities ("Decreto-Lei nº 227-B/2000").

The ecological studies on this species in Portugal are limited to some aspects of its biometrics (Rosalino *et al*., 2005; Bandeira *et al*., 2016) and diet configurations (Rosalino *et al*., 2009; Bandeira *et al*., *unpublished data*). Recently, Bandeira *et al*. (2016) reported that body size of Egyptian mongoose evidenced differences between genders and across regions. To our knowledge, there are no reports elucidating if and how mongoose spleen weight and body condition varies across its distribution range or with differences in seasonal, environmental, ecological and climatic features.

Different populations of species with broad geographic distributions are subject to distinct environmental conditions and community composition (Lucherini *et al*., 2006) which may exert different evolutionary pressures. The presence of distinct prey and competitors also enables contact with different parasites and diseases. In order to survive, the immune system of vertebrates must respond to antigenic challenges, which will irreversibly affect life-history traits and energy allocation trade-offs (Schulte-Hostedde & Gooderham, 2011). The spleen is the primordial secondary lymphoid organ in mammals, playing a key role in immune defense (Corbin *et al*., 2008). It is functionally and histologically divided in to red pulp and white pulp (Mebius & Kraal, 2005). The red pulp ensures blood filtration, removal of effete erythrocytes, regeneration of free ferrous iron, and pathogen clearance (Mebius & Kraal, 2005). The white pulp houses lymphoid components under resting conditions, such as T cells, B cells, and antigen presenting cells (Mebius & Kraal, 2005). Maintaining the immune system is very costly in terms of energy, and individuals with better body condition are likely to have greater capacity for production and storage of lymphocytes (Ponlet *et al*., 2011; Schulte-Hostedde & Elsasser, 2011), which then may translate into heavier spleens. Because it directly influences survival, indicators of immune

competence based on spleen weight and morphology may help to disentangle the factors underlying species success under different environmental scenarios (Vicente et al., 2007).

Spleen mass has been considered as a reliable proxy of individual immunocompetence notwithstanding the other functions that this organ assures (Hosken & O'Shea, 2001; Corbin *et al*., 2008; Hadidi *et al*., 2008; Navarro-Gonzalez *et al*., 2011; Manjerovic & Waterman, 2012). At the intraspecific level, larger spleens may reflect greater investment in immunity for healthy individuals with better body condition (Moller *et al*., 1998*b*), while smaller spleens suggest the opposite (Diez-Leon *et al*., 2013). However, the use of spleen weight to evaluate within species immunocompetence has limitations. Sudden increases in spleen mass due to intense physical exertion and stress (Corbin *et al*., 2008) or an ongoing infection might overweight host-individual traits when assessing immune capacity (Gouy de Bellocq *et al*., 2007). The relative amount of red blood cells stored in the mammalian spleen is known to vary upon stress, exercise or haemorrhagic trauma (Brendolan *et al*., 2007). Alternatively, the enlargement of the spleen may reflect an immune response to parasitism (Nunn, 2002; Gouy de Bellocq *et al*., 2007; Corbin *et al*., 2008), or inflammation due to pathological changes (Moller *et al*., 1998*a*). Finally, while recognizing that the spleen mass is dependent on body condition, it may also be influenced by variation in partitioning efforts among reproduction and growth (Vicente *et al*., 2007).

Body condition refers to the amount of energy reserves that an animal displays, such as fat and protein (Perez-Orella & Schulte-Hostedde, 2005; Schulte-Hostedde *et al*., 2005), and it represents the energetic state of an animal (Schulte-Hostedde *et al*., 2001). Body condition scores have been developed as bioindicators of overall health or physical quality of each specimen (Peig & Green, 2009). Several studies have approached the relationship between body condition of mammals and ecological parameters (Green, 2001), such as the dependence of animal density and fecundity (Stewart *et al*., 2005), parturition and weight of litter (Dobson & Michener, 1995), stress (Young & Monfort, 2009), effect of ectoparasitism (Perez-Orella & Schulte-Hostedde, 2005), or prey selection (Pierce *et al*., 2000).

Considering that Egyptian mongoose is expanding its distribution into new habitats characterized by different environmental gradients (Barros & Fonseca, 2011), we set out to investigate whether the spleen weight and body condition of this species suffers variation with age, gender, season or geographic region. We also investigated if there is a relation

between spleen weight and energy reserves (fat) and body size, or distinct environmental, ecological, human or climatic pressures. Moreover, considering the trends in mongoose biometric measurements that we identified in our previous study (Bandeira *et al.*, 2016), we expected that reproductive months (winter) and gender (male) would exert a positive effect on energy reserves and spleen weight.

3.4. MATERIAL AND METHODS

3.4.1. *STUDY AREA*

Wild Egyptian mongoose specimens were collected from 13 of 17 districts of continental Portugal. Geographic origin of the specimens was attributed to the North or South of the Tagus River, due to marked bioclimatic, biogeographic and anthropic pressure differences previously observed between both regions (Bandeira *et al*., 2016). The Tagus River was considered a geographical barrier for the species, since the distribution of the Egyptian mongoose was more concentrated in the south until last three decades (Borralho *et al*., 1996; Barros & Fonseca, 2011). Regarding land cover and habitat characteristics of each region, flora found in the south is mainly characterized by *Quercus* evergreen species trees, and in the north, the prevailing flora consists of monoculture of *Eucaliptus* sp., which replaced a large part of the areas occupied by *Pinus pinaster* and native deciduous trees (Alves *et al*., 2009).

3.4.2. *SAMPLING PROCEDURES*

Sampling took place between January 2008 and December 2014. Specimens were obtained from hunting activities and road kills, according to legal requirements and under license from competent authorities. Carcasses were labeled with collection date and location and stored at -20°C until the date of processing. In the laboratory, samples were thawed, sexed, weighted, measured and dissected. Spleens were collected and weighted. Measurements collected were snout-tail length (terminal hairs not included), right hind leg length, right

hind foot length, shoulder height, neck perimeter and head width (Bandeira *et al*., 2016). Sampling yielded 678 specimens, but only 518 were selected for this study, for which age determination was possible and an intact spleen was present. Pregnant females were excluded to avoid bias of body weight and body condition variables.

An index of subcutaneous and visceral fat adapted from Braun (2005) was used as a proxy for energy reserves. Fat measurements were performed with a hand caliptometer. Index 0 was attributed to animals without fat, index 1 to animals with vestigial fat measuring 0.5 mm or less, index 2 to animals with vestigial fat measuring between 0.5 mm and 2 mm, index 3 to animals with a thick fat layer, and finally, index 4 was attributed to animals with a continuous layer of subcutaneous fat (measured on sternum) or kidneys covered with visceral fat. Age was determined by dentition analysis. Each specimen was assigned to one of four age classes: adults over one year of age, sub-adults between nine and twelve months, juveniles type II between five-and-a-half and nine months, and juveniles type I between two-and-a-half and five-and-a-half months of age, depending on the level of development of its dentition (see Bandeira *et al*., 2016). Individuals collected between January and March were considered as individuals collected in winter; in spring, between April and June; summer, between July and September; and autumn, between October and December.

3.4.3. *ENVIRONMENTAL AND ECOLOGICAL DRIVERS*

Based on ecological requirements and physiological characteristics of Egyptian mongoose, 20 environmental variables that could directly or indirectly influence body condition and spleen weight were selected for modelling (Table 3.1) (eg. Ben-Yaacov & Yom-Tov, 1983; Delibes *et al*., 1984; Palomares & Delibes, 1990; Barros *et al*., 2015; Bandeira *et al*., 2016).

Each variable was represented by mean values of the 2 x 2 km grid cell, considering the vital area of the Egyptian mongoose (Palomares & Delibes, 1991*b*).

Table 3.1. Mean, maximum and minimum values of environmental and ecological variables.

Variable	Mean value	Maximum value	Minimum value	Source
Urban (ha)	12.49	218.60	0	
Rice fields (ha)	2.95	260.83	0	
Agro-forestry (ha)	96.43	317.22	0	
Shrubs (ha)	64.92	297.29	0	
Inland Water Bodies (ha)	1.03	125.26	0	(Corine Land Cover, 2006)
Vineyards & Orchards (ha)	6.17	339.51	0	
Coniferous (ha)	8.74	143.18	0	
Broadleaved & Mix forests (ha)	86.70	380.99	0	
Agriculture (ha)	109.94	400	0	
Altimetry (m)	199.64	976.91	8.70	(ASTER, 2015)
Human population density (hab./km^2)	97.15	2477.51	0	(European Comission, 2015)
Road network (m)	6917.45	66751.47	0	(IGP, 2015)
River network (m)	1929.57	5199.00	0	(SNIRH, 2015)
Average annual temperature (°Cx10)	162.59	170.67	108.83	
Annual temperature range (°Cx10)	244.78	273.17	150.50	(BioClim, 2015)
Annual rainfall (mm)	654.70	1479.50	486.83	
NDVI	0.50	0.79	0.23	(MODIS, 2015)
*Egyptian mongoose abundance proxy (x animals/400ha)	3.34	10.88	0.03	
*European rabbit abundance proxy (x animals/400ha)	98.97	921.13	0	(ICNF, *unpublished data*)
*Red-legged partridge abundance proxy (x animals/400ha)	32.22	208.05	0.23	

* Number of animals hunted in each area and during the month, where and when, each Egyptian mongoose sampled was collected.

Climate data comprised three variables gathered from BioClim (2015) at 30 arc-second resolution (Hijmans *et al*., 2005). Nine variables (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests and agriculture areas) accounting for habitat structure were retrieved from Corine Land Cover (2006) with a spatial resolution of 250 m. The Normalized Difference Vegetation Index (NDVI, hereafter) was used as a proxy of primary productivity (Pettorelli, 2013). NDVI values for each record was calculated from satellite images supplied by Moderate Resolution Imaging Spectroradiometer (MODIS, 2015) at a spatial resolution of 250 m. The elevation data was computed using the ASTER Global Digital Elevation Model platform (ASTER, 2015). Hydrographic data were gathered from the Sistema Nacional de Informação de Recursos Hídricos (SNIRH, 2015). The extent of human-related effects was investigated using two variables: population density [derived from the European Commission (2015)] and road network (IGP, 2015). Latitude and longitude were used as a measure of geographic position of the collected samples. Ecological variables included Egyptian mongoose (*Herpestes ichneumon*), European rabbit (*Oryctolagus cuniculus*) and red-legged partridge (*Alectoris rufa*) abundances based on annual hunting yields (ICNF, *unpublished data*).

3.4.4. *STATISTICAL PROCEDURES*

All variables were tested for normality with Kolmogorov-Smirnov Test (with correction of Lilliefors for the significance level) (Zar, 1999).

The Principal Component Analysis (PCA) enables a single estimate of body size on the first component, based on the covariance matrix of various measures. Body size was calculated by combining weight and the six biometric measurements into a single value through a principal component analysis (PCA), using all variables with loadings higher than 0.70. Spleen weight was corrected for body weight by presenting spleen weight (in grams) per 100 grams of total mass of the animal (adjusted spleen weight). The body condition score was calculated as the values of standardized residuals of a regression between total body mass and snout-tail length (eg. Dobson & Michener, 1995; Schulte-Hostedde *et al*., 2001; Blackwell, 2002; Cattet *et al*., 2002; Schulte-Hostedde *et al*., 2005;

Stevenson & Woods, 2006), whereby positive values indicate a good body condition and negative values indicate a poor body condition (Blackwell, 2002).

The effect of age, gender, region and season on spleen weight and on body condition scores was analyzed by means of Generalized Linear Mixed Models (GLMM) where the district (first-level administrative subdivision of mainland Portugal, dimensioned based on history, common land use and related issues) was used as a random factor to control for non-independence of samples from the same area. Only *p* values lower than 0.05 were considered statistically significant. In the case of spleen weight, the continuous variables studied were subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, landscape structure, altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, NDVI, body size and body condition. In the case of body condition scores, the same variables were used in GLMM except the body condition itself, which was then replaced by spleen weight.

Following the identification of significant variables, a selection of models explaining spleen weight variation was performed according to the procedure described in Zuur *et al*. (2009). A ranking was made of all possible models using the Akaike Information Criterion (AIC) (Burnham & Anderson, 2002) and only those with differences in values of AICc lesser than 2 were considered explanatory. The same procedure was applied to obtain models for the variation in body condition scores. If several models were similar to explain data, then an averaging modelling approach was used (Burnham & Anderson, 2002). The relative importance of variables was also estimated through Akaike weights (Burnham & Anderson, 2002).

All statistical analyses were performed using R (version 2.13.2), using the R-package *lme4* (Bates *et al*., 2014) and *MuMIn* for multimodel-selection and modeling averaging approaches (Barton & Barton, 2015).

3.5. RESULTS

A total of 518 Egyptian mongooses were included in this study, 272 females and 246 males. There were 269 adults, 85 sub-adults, 95 type II juveniles and 69 type I juveniles (Table 3.2). Regarding geographic origin, 135 and 383 specimens came from north and south of Tagus River, respectively (Figure 3.1, Table 3.2).

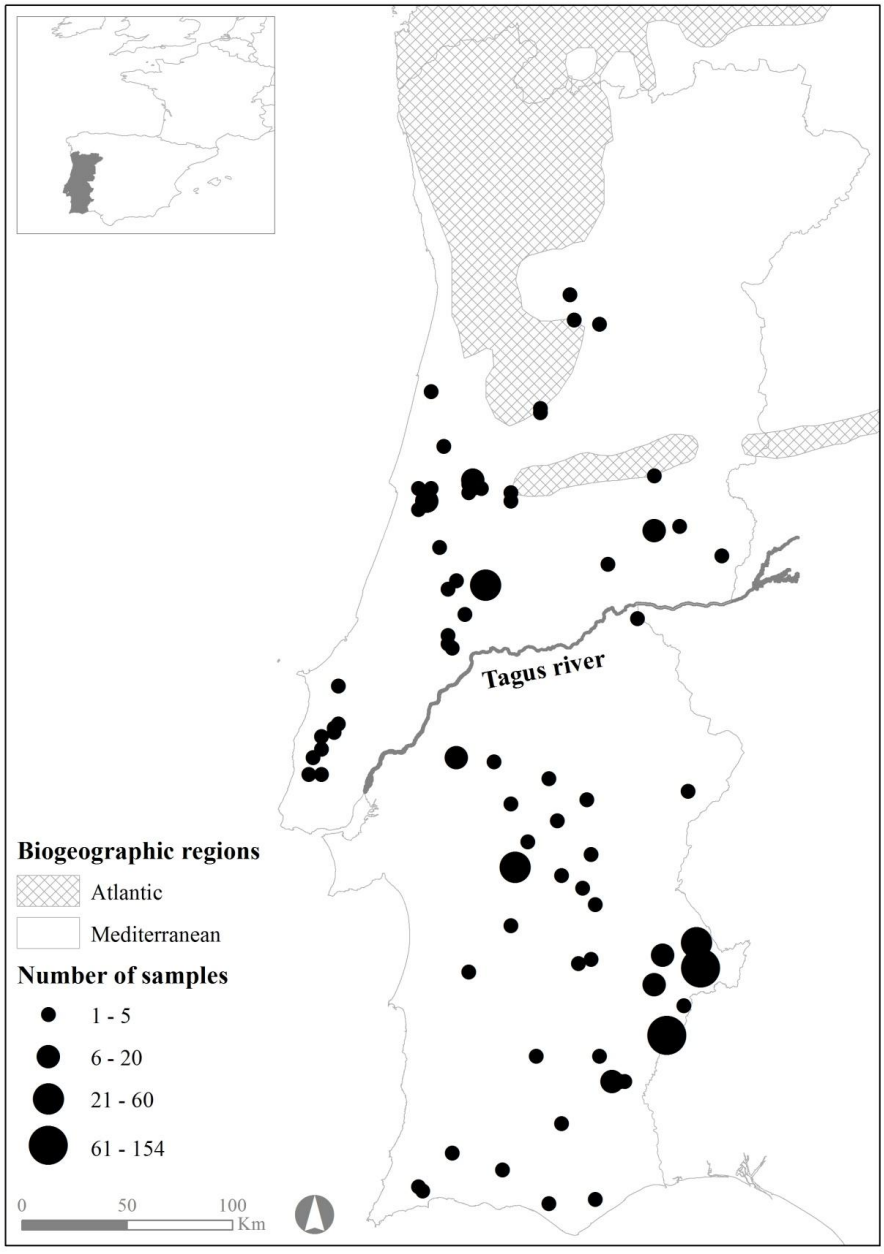

Figure 3.1. Locations and number of samples of the Egyptian mongoose specimens under study.

Table 3.2 Number of Egyptian mongoose samples obtained for each region, north and south of Tagus River, within age class and gender information.

Age	Gender	Region	
		North	South
Adult	Female	43	108
	Male	36	82
Sub-adult	Female	13	33
	Male	16	23
Juvenile II	Female	5	34
	Male	9	47
Juvenile I	Female	4	32
	Male	9	24
Total	Female	65	207
	Male	70	176

PCA of body size for each specimen was explained by a proportion of variance of 74.6%, with an eigenvalue of 5.220. The body condition adopted the values of standardized residuals from the total body mass against total body length regression [$F(1,516)=978.22$, $p<0.001$].

The GLMM analysis on adjusted spleen weight variation evidenced effects of season ($p < 0.05$), Egyptian mongoose abundance ($p < 0.05$), European rabbit abundance ($p < 0.05$), red-legged partridge abundance ($p < 0.05$), annual rainfall ($p < 0.05$), body condition ($p < 0.05$), and of the interaction between gender and age class ($p < 0.05$) (Table A2.1, data as supplementary table).

After ranking all possible models using AICc, only one model lower than 2 was obtained and considered explanatory (Table 3.3). It included season + gender, with an AICc of -153.70, (df=517) (Tables 3.3 and 3.4). Males presented higher spleen weight per 100 g of body weight than females, and regarding season, spleens were heaviest in winter, followed by spring, and then autumn, and were lightest in summer (Table 3.4). To observe detailed information on each factor, refer to Figures 3.2 and 3.3.

Table 3.3 Model selection using AICc for the effect of age, region, season, gender, and their interactions, Egyptian mongoose, European rabbit and red-legged partridge abundances, annual rainfall and body condition, with district as random factor, on spleen weight (expressed as g/100g body weight) of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df - degrees of freedom.

Models	**df**	**AICc**	**ΔAICc**	**AICc weight**
SEASON + GENDER	7	-153.49	0.00	0.74
SEASON + REGION + GENDER	8	-151.45	2.04	0.26

Table 3.4 Effects of the model considered as explanatory for spleen weight (expressed as g/100g body weight) of the Egyptian mongoose in Portugal.

Variables		**Estimate**	**Std. Error**	**t value**
Intercept		0.350	0.039	9.020
GENDER	Male	0.069	0.018	3.928
	Spring	0.073	0.024	3.057
SEASON	Summer	-0.037	0.024	-1.522
	Winter	0.083	0.027	3.024

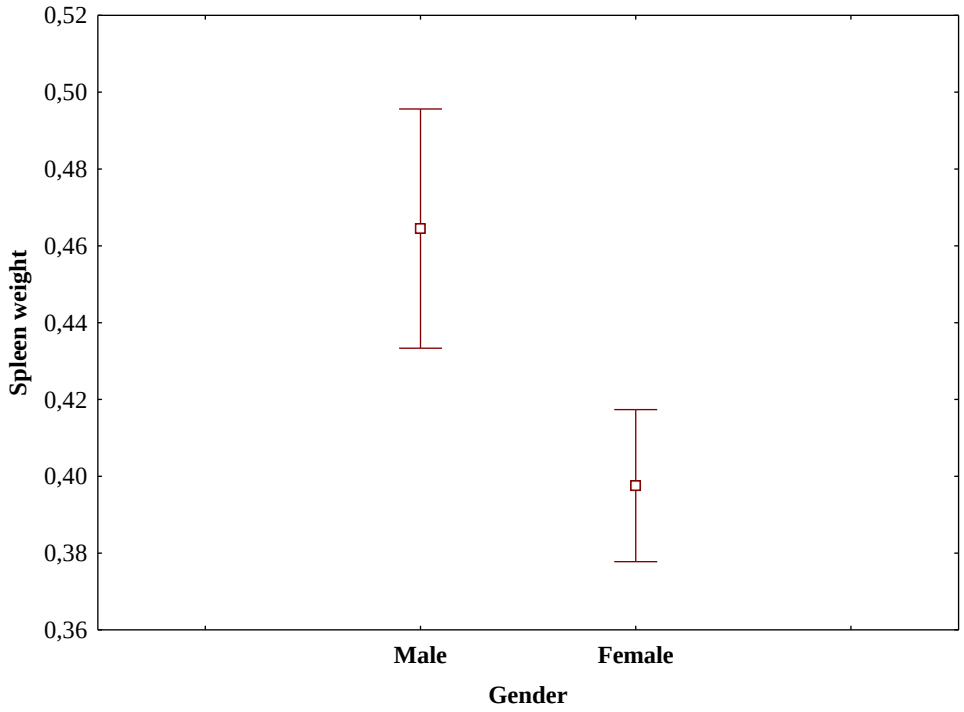

Figure 3.2 Means of Egyptian mongoose spleen weight (expressed as g/100 g body weight) observed for each gender. Vertical bars denote 95% confidence intervals.

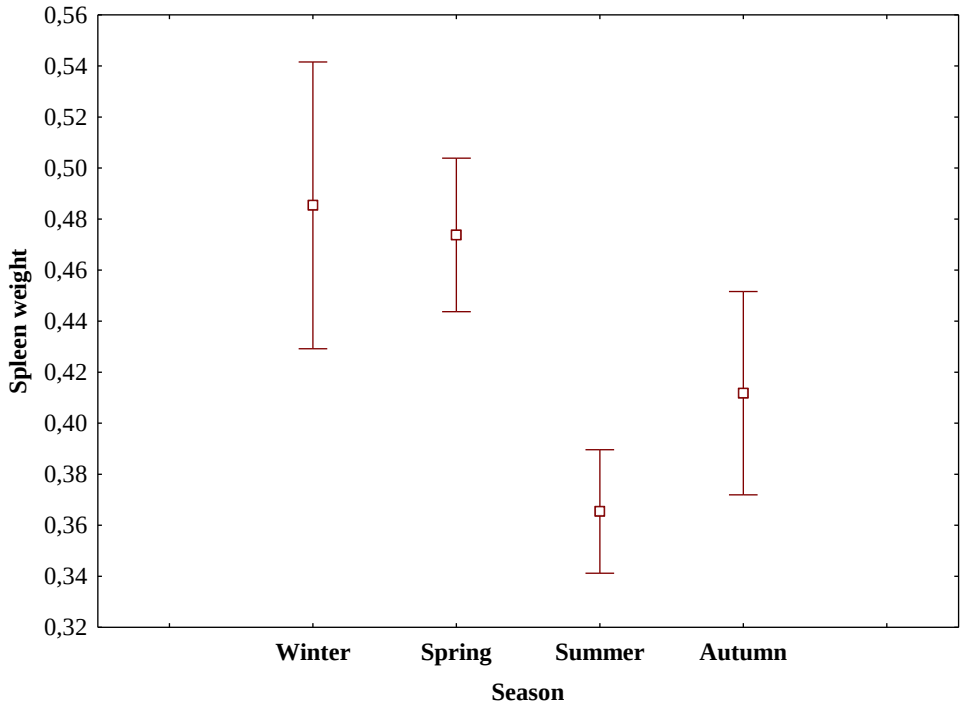

Figure 3.3 Means of Egyptian mongoose spleen weight (expressed as g/100 g body weight) observed for each season. Vertical bars denote 95% confidence intervals.

The GLMM analysis on body condition variation showed effects of season ($p < 0.05$), age class ($p < 0.05$), spleen weight ($p < 0.05$), subcutaneous fat ($p < 0.05$) and visceral fat ($p < 0.05$) (Table A2.2, data as supplementary table).

After ranking all models with AICc, two models were considered explanatory (Table 3.5). The first included spleen weight + visceral fat + subcutaneous fat + age + gender, and the second model included spleen weight + visceral fat + subcutaneous fat + age, with AICc's of 1323.98 and 1324.90, respectively, and df=517 (Tables 3.5 and 3.6).

Table 3.5 Model selection using AICc for the effect of age, region, season, gender, and their interactions, spleen weight (expressed as g/100 g body weight), subcutaneous fat and visceral fat, with district as random factor, on body condition (residuals from the regression of total body weight against total body length) of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df - degrees of freedom.

Models	**df**	**AICc**	**ΔAICc**	**AICc weight**
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE + GENDER	10	1323.98	0.00	0.41
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE	9	1324.90	0.92	0.26
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE + REGION + GENDER	11	1326.06	2.08	0.14
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE + REGION	10	1326.89	2.91	0.10
SPLEEN WEIGHT + SUBCUTANEOUS FAT + AGE + GENDER	9	1328.06	4.08	0.05
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE + REGION + GENDER + REGION × GENDER	12	1328.47	4.49	0.04

Table 3.6 Models considered as explanatory on body condition (residuals from the regression of total body weight against total body length) of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	**df**	**AICc**	**ΔAICc**	**AICc weight**
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE + GENDER	10	1323.98	0.00	0.61
SPLEEN WEIGHT + VISCERAL FAT + SUBCUTANEOUS FAT + AGE	9	1324.90	0.92	0.39

Because two models were selected, averages of an approximate ensemble model were used (Tables 3.7). The variables with most relative importance were spleen weight, visceral fat, subcutaneous fat and age (Table 3.7). Males presented higher body condition scores than females (Table 3.7); adult Egyptian mongooses were in better body condition compared to juveniles type I, sub-adults and juveniles type II, in descending order, respectively (Table 3.7). Specimens with better body condition presented higher levels of subcutaneous and visceral fat and heavier spleens (Table 3.7).

Table 3.7 Model-averaged coefficients for the effects of SPLEEN WEIGHT, VISCERAL and SUBCUTANEOUS FAT, AGE and GENDER on body condition (residuals from the regression of total body weight against total body length) of the Egyptian mongoose in Portugal.

Variables		**Coefficient**	**Std. Error**	**Z value**	**Relative importance**
Intercept		-0.933	0.132	7.080	
SPLEEN WEIGHT		0.721	0.185	3.907	1.00
VISCERAL FAT		0.208	0.066	3.174	1.00
SUBCUTANEOUS FAT		0.246	0.059	4.158	1.00
AGE	Juvenile 1	-0.216	0.115	1.876	1.00
	Juvenile 2	-0.823	0.105	7.827	
	Sub-adult	-0.378	0.104	3.621	
GENDER	Male	0.192	0.076	2.529	0.61

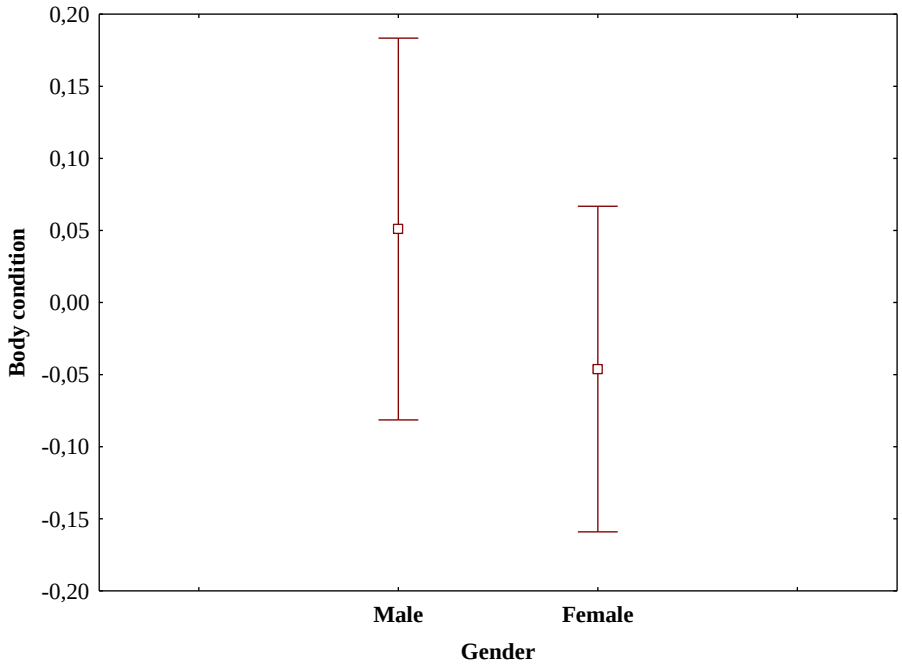

Figure 3.4 Means of Egyptian mongoose body condition (residuals from the regression of total body weight against total body length) observed for each gender. Vertical bars denote 95% confidence intervals.

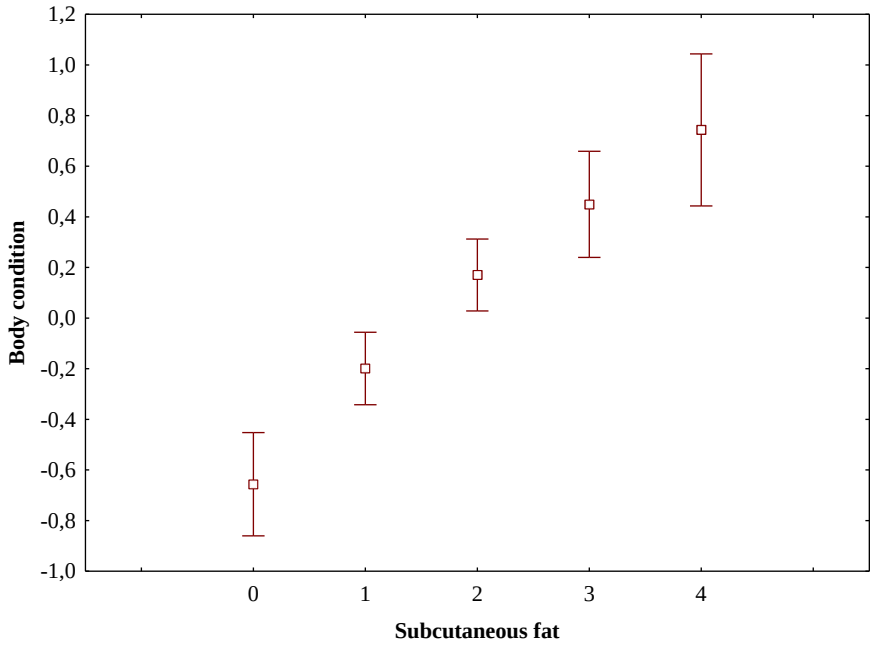

Figure 3.5 Means of Egyptian mongoose body condition (residuals from the regression of total body weight against total body length) observed for subcutaneous fat indexes (ranging 0-4). Vertical bars denote 95% confidence intervals.

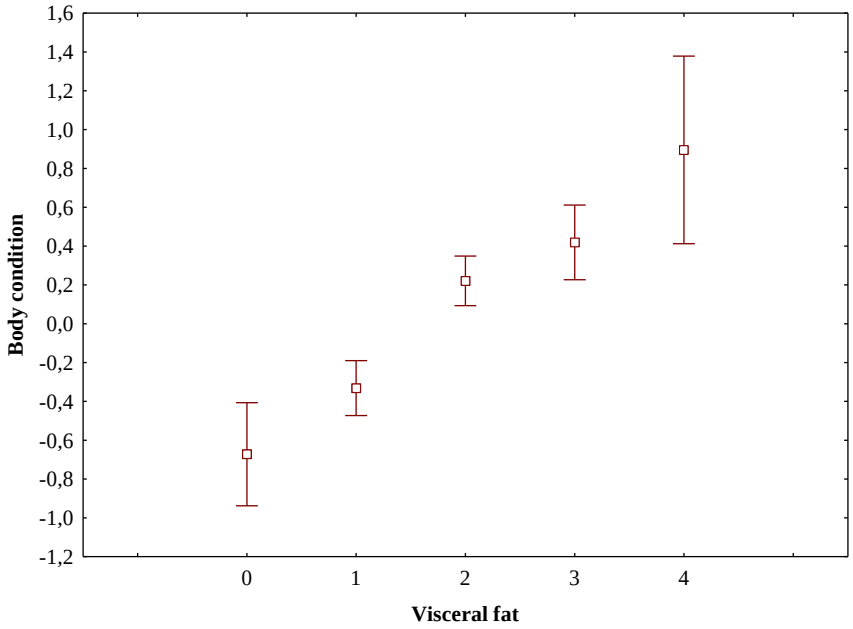

Figure 3.6 Means of Egyptian mongoose body condition (residuals from the regression of total body weight against total body length) observed for visceral fat indexes (ranging 0-4). Vertical bars denote 95% confidence intervals.

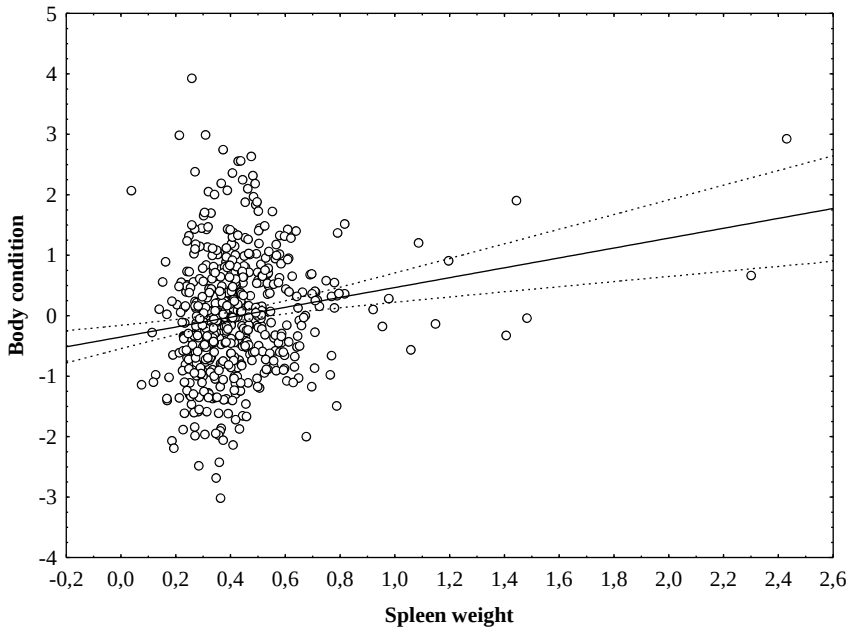

Figure 3.7 Scatterplot of Egyptian mongoose body condition (residuals from the regression of total body weight against total body length) observed for spleen weight (expressed as g/100 g body weight). Dashed lines denote 95% confidence intervals. Body condition = -0.3508+0.817*spleen weight.

3.6. DISCUSSION

The analysis of a large sample of free-ranging Egyptian mongoose from almost the entire country evidenced that male Egyptian mongooses have better body condition than females, which contrasts with other studies where no gender differences were reported for dimorphic carnivores (*Canis latrans*, Windberg *et al*., 1991; *Acinonyx jubatus jubatus*, Marker & Dickman, 2003). The current information on the diet of mongoose indicates that males ingest prey with higher energetic value (Rosalino *et al*., 2009; Bandeira *et al*., *unpublished data*), which could explain more energy reserves in this gender. Males are territorial and have larger territories than females (Palomares & Delibes, 1993*b*; Palomares, 1994), which implies covering greater distances and having to fend off opponents, favoring those in better physical condition (Marker & Dickman, 2003). Males also engage in aggressive behavior with females during mating (Palomares, 1991) and therefore a better physical condition may also increase their ability to achieve a successful copulation.

According to our findings, adults exhibited better body condition compared with other age groups. Interestingly, among the three younger age groups, juvenile in the early stage (type I) had the best body condition. During this stage, juveniles are completely dependent on food provided by mother, while in the next phase, type II juveniles already capture their own prey, therefore relying on their own skills (Ben-Yaacov & Yom-Tov, 1983). In addition, competition and disputes for prey (Palomares, 1991) may increase within the litter as they grow. The reliance on their own hunting skills, combined with increased level of competition within the litter, may explain why Egyptian mongooses juvenile phase II exhibited a decrease in body condition in relation to type I juveniles.

Regarding the interaction between body fat and body condition indexes, larger energy reserves in the form of subcutaneous or visceral fat predicted better body condition, which was already expected, since the body condition is reflective of the energetic state (Schulte-Hostedde *et al*., 2001).

The adjusted spleen weight of male mongooses was found to be significantly higher than females', which is in agreement with a previous study on red deer (see Corbin *et al*., 2008).

However, further research is necessary to determine the causal relations that underlie this variation. We speculate that different reproductive strategies, gender specific behavior and hormonal variations may be related to spleen weight variation, especially since the Egyptian mongoose is a polygynic species (Palomares, 1993*a*), with different investments in reproduction and survival between genders (Palomares, 1993*a*; Palomares & Delibes, 1993*b*). These differences could then translate into a different need or ability of males and females to invest energy and resources in spleen overall function. For example, males defending their territories have greater probability to fight and suffer injury, resulting in augmented blood influx and turn-over of effete erythrocytes in the spleen, which then may reflect on spleen mass. In addition to reproductive behavior, diet may also account for gender differences in spleen weight. The capture of larger and more energetic prey by males (Rosalino *et al*., 2009; Bandeira *et al*., *unpublished data*) may enable more energy reserves and investment in immunity, admitting that spleen weight is a reliable proxy for immunocompetence.

To our knowledge, this is the first time that spleen weight measurements from all year round are presented for a wild carnivore species. Our data collection shows that adjusted spleen weight varies across seasons and that season directly influences spleen weight. In winter, the Egyptian mongoose specimens sampled in our study presented the highest spleen weights, with a slight decrease in values during spring, and an abrupt decrease in summer, followed by a rise again in autumn. A study with wild American mink males (*Neovison vison*) (Persson *et al*., 2011) and another report focused on wild boar (*Sus scrofa*) (Fernandez-Llario *et al*., 2004), showed that animal spleens were heaviest during winter, although both studies only compared data from three or two seasons, respectively. These findings in a carnivore and in an ungulate species are in accordance with our observations in the mongoose.

According to a study on the reproductive parameters of the Egyptian mongoose in Spain, the courtship and the copulations begin in winter and extend almost to the end of spring (Palomares & Delibes, 1992). During the breeding season, the Egyptian mongoose engage in physical aggression to defend their territory to gain access to more females, due to mating behavior, or due to competition for prey (Palomares, 1991; Palomares, 1993*a*). Male investment in immune function may cause a trade-off during reproductive period,

since energetic resources should be directed towards reproduction and less toward the immune response (Zuk & Stoehr, 2002; Stoehr & Kokko, 2006). However, our results do not support this idea because we found that during the reproductive period the spleen weights are higher. Surprisingly, age was not significantly associated with spleen weight in the explanatory model and the positive influence of winter on spleen weight was observed across all age groups, including juveniles. This result seems to contradict the hormone and reproductive related explanations for spleen weight variation and deserves further investigation in the future. It should however be considered that also during the winter, when juveniles reach the dispersion period and approach independence (Palomares & Delibes, 1998), they often suffer aggression from their mothers (Palomares, 1991), which could then trigger immune responses of juveniles to possible damage inflicted by the claws or teeth of the progenitor.

It is also during the winter that animals are more exposed to new antigens (Fernández-Llario *et al*., 2004), which may also contribute to enlargement of the spleen. Parasitism could support spleen weight differences as parasite-infected individuals are expected to have larger spleen sizes due to increased lymphocyte production and/or pathological inflammation (Møller *et al*., 1998*a*). It is assumed that pathogen infection and infestation with parasites in polygynous species may be more associated to one gender (Moore & Wilson, 2002), with males more likely to have higher parasites burdens and infectious agents than females (Moore & Wilson, 2002; Perez-Orella & Schulte-Hostedde, 2005). This association has been attributed to the effects of testosterone reducing immune function (Folstad & Karter, 1992), or to reproductive strategies, as sexual selection and competition (Zuk, 1990). Although indicators of parasite burdens were not available in our study, this assumption corroborates our observations of heavier spleen weights of male mongooses. However, contradictory results have also been reported in the literature; for instance, Vicente and collaborators (2007) found a negative association of spleen size with nematode load in adult red deer males.

It also appears from our modelling efforts that heavier spleens predict a better body condition in mongoose. The spleen depends on the energetic status of the animal and its functioning is energetically costly, so an individual with good body condition should be more able to invest in lymphocyte production and storage of red blood cells (Schute-

Hostedde & Elsasser, 2011). Spleen weight has also been found to be positively correlated to body condition in other mammals, such as in American mink (*Neovison vison*) (Schute-Hostedde & Elsasser, 2011), or red deer (*Cervus elaphus*) (Vicente *et al*., 2007). On the other hand, parasitism normally has negative consequences on body condition, because parasites extract energy resources from the host (Neuhaus, 2003). Furthermore, parasitism can also stimulate the immune activity (Navarro-Gonzalez *et al*., 2010; Schute-Hostedde & Elsasser, 2011), and then energy is allocated to the immune system rather than being directed to growth and maintenance of body condition (Perez-Orella & Schulte-Hostedde, 2005). We cannot test for the influence of parasite diversity or burden due to the lack of data. However, if spleen weight was to be related with parasitism, we would expect it to be associated with poorer body condition, rather than with increased body condition scores.

Since ecological conditions of the Egyptian mongoose from south of the Tagus River are more favorable than those from north (see Bandeira *et al*., 2016), we expected geographic and seasonal differences, as well as gender dimorphism, to influence spleen weight and body condition, presumably originating heavier spleens and better condition indexes in the South. The fact that the spleen weight and body condition values do not vary under the action of any of the environmental or climatic variables analyzed in this study supports the hypothesis that the capacity for immune response and the energetic state depend more directly on causes related with gender, diet, reproductive behavior, and antigenic challenge, rather than the influence of habitat, primary productivity, rainfall or temperatures. As this is a generalist species in terms of habitat (Palomares & Delibes, 1990; Palomares & Delibes, 1993*a*) and diet (eg. Delibes *et al*., 1984; Palomares & Delibes, 1991a; Palomares, 1993*b*; Rosalino *et al*., 2009), we propose that mongoose behavioral and dietary plasticity result in little influence of environmental conditions on spleen weight, and thus immunocompetence, and on body condition, which in turn appear to be mostly influenced by life-history traits and gender-specific differences.

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CHAPTER 4

DIET

4.1. Diet footprint of Egyptian mongoose along ecological gradients: effects of primary productivity and life-history traits

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4.2. ABSTRACT

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is an expanding species, whose presence in Europe is restricted to its western extreme. Mongooses are generalist and opportunistic carnivores which is reflected by their anatomical features and nutritional preferences. This game species in Portugal has a negative connotation within the hunting community that imputes it to depredate mostly on small game species with great economic interest. With the purpose to fully characterize mongoose diet and ascertain if game species overlap within the guild of consumed food items, a total of 122 stomach contents of animals resulting from hunting activities and accidental road kills from seven provinces of mainland Portugal were identified. In order to identify which factors influence the nutritional requirements and food preferences of the Egyptian mongoose, several physiologic parameters (body size and condition, spleen weight, age class, fat levels and gender) were related with bio-ecologic features, as seasonality, region, habitat primary productivity, climate, habitat pressure and environmental conditions.

The results suggested that the diet of the Egyptian mongoose is mainly composed of mammals, reptiles, amphibians and invertebrates. Mammals and amphibians were predominant in males' stomach contents, while reptiles and invertebrates overlapped other food categories in females' diet. Diet (food categories) variations across age groups and regions were registered. The consumption of prey also varied throughout the year, according to availability and mongoose energy uptake needs, with a higher consumption of more energetic prey during the breeding season. Most prey categories were consumed in places with higher primary productivity, with the exception of reptiles that were mostly consumed in the drier areas. Our data thus suggests that the consumption of more energetic prey, such as mammals, results in better body condition and heavier spleens, possibly driving to greater investment in immunity and better animal performance.

Overall, we show that the ingestion of game species, although relevant, represents less than half of the trophic ecology of Egyptian mongoose, which proved to be quite biodiverse. The exploratory and digging behavior of this species, and minimal energy expenditure in search of the most abundant and vulnerable prey, seem to take advantage of the different periods of less physical mobility, but of greater ecological activity of each prey category.

Furthermore, we show that sexual dimorphism in the use of ecological niche exerts downstream effects that ultimately translate into a gender-specific ecological footprint of the species.

KEYWORDS

Herpestes ichneumon; carnivore; Iberian Peninsula; food item; biomass consumed; stomach contents; trophic ecology.

4.3. INTRODUCTION

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a medium-sized carnivore that is in expansion due to the change of land use and climate variations over time in Portugal (Barros & Fonseca, 2011; Barros *et al*., 2015), and in Spain (Talegón & Parody, 2009; Recio & Virgós, 2010; Balmori & Carbonell, 2012). This game species in Portugal (regulated by laws "Decreto-Lei nº 227-B/2000"; "Decreto-Lei nº202/2004" with the new wording given by "Decreto-Lei nº 201/2005") is negatively perceived by hunters, who consider it responsible for the predation of their most important small game species, which include the European rabbit (*Oryctolagus cuniculus*) and the red-legged partridge (*Alectoris rufa*). Scientific studies describe Egyptian mongoose as an opportunistic and generalist predator, capturing more abundant, available and vulnerable prey, according to occasion (Delibes *et al*., 1984; Palomares & Delibes, 1998). None of the available studies focuses on the prey percentages of game species consumed by mongooses, failing to address the hunting community's concerns.

Previous work has shown that, in Portugal, the Egyptian mongoose populations exhibit sexual dimorphism in body size, in which adult males are significantly larger than adult females (Bandeira *et al*., 2016). These same adult males have heavier spleens and higher energy reserves (Bandeira *et al*., *submitted*). Since males capture larger and more energetic prey (Rosalino et al. 2009) and may enable more energy reserves and investment in immunity (inferred from spleen mass as proxy of immune competence) (Bandeira et al., *submitted*), diet could be one of the drivers of these dimorphisms.

A geographically-limited study of the diet of this mongoose species in Portugal showed a variation along season and between gender, wherein females were found to include more reptiles on their diet, while males consumed more mammals, also reporting that during the winter and spring more mammals were consumed (Rosalino *et al*., 2009). In a regional study conducted in Serra de Grândola (SW Portugal), a large consumption of mammals was recorded, with mongoose diet also including fruits, birds, invertebrates and reptiles (Santos *et al*., 2007). Rosalino *et al*. (2010) reported that the fruits consumed corresponded to spontaneous species in the region while Santos *et al*. (2007) found that prey were not consumed according to availability, suggesting some preferences for some food resources.

In Spain, the Egyptian mongoose diet, although not divergent between genders, varies locally and seasonally depending on the availability of prey (Delibes *et al*., 1984; Palomares & Delibes, 1991*a*). In the region of Doñana (Southwest Spain), the Egyptian mongoose was found to be opportunist, consuming the most abundant prey in each area and season (Palomares, 1993). In the above studies, some young rabbits were recorded as captured within their own burrows (Palomares, 1993). Even with the decrease of rabbit populations caused by viral hemorrhagic fever, its consumption by mongooses was apparently not affected as with other carnivores (Ferreras *et al*., 2011). Likewise, rabbit intake was also registered during myxomatosis outbreaks (Delibes & Beltran, 1985). Further south, in the Doñana Biological Reserve, the diet composition seems to differ due to the lower consumption of rabbits compared to the other food categories (Palomares & Delibes, 1991*a*).

In Israel, where the species is also present, a quantitative study on the consumed items was not carried out, but some components were identified to determine diet diversity and habitats where Egyptian mongoose capture prey (Ben-Yaacov & Yom-Tov, 1983). In this region of the Mediterranean basin, the Egyptian mongoose is known to predate snakes, despite poisonous (Ben-Yaacov & Yom-Tov, 1983), because it is resistant to the most toxic components of the elapid and viperid venoms (Bdolah *et al*., 1997). In Egypt, few components of the trophic ecology of the Egyptian mongoose have been identified (Basuony *et al*., 2013).

For the Egyptian mongoose, prey availability seems to be the most important factor in habitat choice (Palomares & Delibes, 1990). Riparian galleries, especially in Mediterranean environments, are very important habitats for carnivore species richness in all landscape contexts, because apart from water and food, they provide anti-predator shelter (Virgós, 2001; Santos *et al*., 2011). These places are especially important for Egyptian mongoose, since it avoids open areas, prefers dense vegetation indices, with less frequent fire and burnt shrub, that consequently provide higher densities of prey (Palomares & Delibes, 1990; Palomares & Delibes, 1991*a*; Palomares & Delibes, 1993). Since diet composition of a resident species is promoted by high prey abundance or primary productivity (Lozano *et al*., 2006; Zhou *et al*., 2011), the availability and

consumption of prey is expected to be higher in the Egyptian mongoose's territories with higher primary productivity.

Trophic ecology has been widely investigated in many species of carnivores (eg. Cavallini & Volpi, 1996; Beja, 1997; Fedriani *et al*., 1998), because their position at the top of the food chain may affect the distribution and abundance of other species (Chuang & Lee, 1997), as well as their own ecology. The variation of diet between genders (eg. Erlinge, 1981; Cavallini & Volpi, 1996; McDonald *et al*., 2000; Zalewski, 2007), across the seasons (eg. Beja, 1997; Fedriani *et al*., 1998; Rosalino & Santos-Reis, 2002; Carvalho & Gomes, 2004) and between regions (Cavallini & Volpi, 1996; Virgós *et al*., 1999) has been reported for several species of carnivores. In Mediterranean landscapes, there are resources that are not consistently found throughout the year, such as fruits, arthropods, reptiles or amphibians, creating the temporary need for other available food sources (Virgós & Casanovas, 1999; Rosalino *et al*., 2005). Thus, the seasonal variation of diet is related with prey availability (Cavallini & Volpi, 1996; Chuang & Lee, 1997; Rosalino & Santos-Reis, 2002).

With this study in particular, we aim at characterizing mongooses' diet using a collection of specimens for which a series of accessory biological information was also gathered (spleen weight, body size and body condition). Connecting our own generated data with food preferences and energy requirements of the species will hopefully shed light into the ecology of this carnivore. We took this opportunity to understand whether and how the primary productivity of habitats, the spleen weight, body size and condition influence diet variation of the Egyptian mongoose, between gender and age class, and throughout the seasons and from region to region. We suppose that males consume more energetic prey, and that this consumption increases proportionally with age. We conjecture that the consumption of different prey categories varies geographically and seasonally. We presume that animals with better condition and body size, greater spleen weight and living in areas with higher primary productivity, will capture and consume larger and more energetic prey. In contrast, animals with divergent biological parameters will invest in the intake of less energetic prey, which require less effort in capture and manipulation. Finally, we aim to answer if game species are indeed the most commonly consumed prey by Egyptian mongoose, to clarify the general doubts within the hunting community.

4.4. MATERIAL AND METHODS

4.4.1. *STUDY AREA*

This work gathered wild Egyptian mongoose from seven of the nine provinces (dimensioned based on history, common land use and related issues) of mainland Portugal, where the species is present. According to the origin of the sample, one of two regions were assigned to each specimen - north or south, divided by the Tagus River, which was considered as a geographical barrier, taking into account that the species was more concentrated in south until the 90s of the last century (Barros & Fonseca, 2011; Barros *et al*., 2015). This geographic barrier divides the two regions that differ in terms of ecological, climatic and human pressure parameters (Bandeira *et al*., 2016). Regarding land use of each region, flora found in the south is mainly characterized by *Quercus suber*, also with *Olea europaea* and *Quercus ilex* areas, where agroforestry ("montado") and shrubs habitats predominate (Alves *et al*., 2009), with higher temperatures and lower levels of rainfall (Bioclim, 2015). In the north, the prevailing flora consists of monoculture of *Eucaliptus* sp., which replaced a large part of the areas occupied by *Pinus pinaster*, *Quercus robur*, *Salix* sp. or *Alnus glutinosa* (Alves *et al*., 2009). Here, human pressure is higher, with more villages and more population density, more kilometers of roads, more fragmented habitats with greater variability (Alves *et al*., 2009; European Commission, 2015; IGP, 2015). It is also noted that northern of the Tagus there are a greater number of ridges and kilometers of hydrographic network (SNIRH, 2015).

The following ecological and climatic variables were represented as mean values for 2 x 2 km grid, considering the vital area and provenance of each collected mongoose (Palomares and Delibes, 1991*c*). Thus, Egyptian mongoose, European rabbit and red-legged partridge abundances reflect means of captured animals by each grid, during the respective collection year, through hunting activities (ICNF, *unpublished data*). The values attributed to each kind of habitat present in each grid (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests and agriculture areas) refer to the number of occupied hectares (Corine Land Cover, 2006). The altimetry values allude to mean in meters per grid (data SRTM, NASA, resolution of 30 meters, computed using the ASTER Global Digital Elevation Model platform) (ASTER,

2015). The human population density values remit to the number of inhabitants per km^2 in each cell (data from Eurostat per kilometer) (European Commission, 2015). The road and river network values refer to the distance in meters of road (IGP, 2015) and hydrographic network (SNIRH, 2015), respectively. The average annual temperature and annual temperature range values allude to temperature in Celsius degrees multiplied by 10, and annual rainfall values were presented in mm per cell (Hijmans *et al*., 2005; BioClim, 2015). Finally, Normalized Difference Vegetation Index (NDVI), which reflects means of primary productivity (Pettorelli, 2013), whose values for each record was calculated at a spatial resolution of 250m from satellite images supplied by MODIS (2015) (Moderate Resolution Imaging Spectroradiometer).

4.4.2. *SAMPLING PROCEDURES*

Between January 2008 and December 2014, 678 Egyptian mongoose carcasses were collected from hunting activities or accidental road kills, and their use was approved by national authorities. The carcasses were labeled with the date and place of collection, and frozen at -20°C. For the present study, we used the stomach contents of 122 animals. Animals without stomach contents or whose content was only composed of bait used in hunting activities were discarded. Individuals whose age could not be determined by dentition were rejected.

Thawed carcasses were sexed, weighed, measured [snout-tail length (terminal hairs not included), right hind leg length, right hind foot length, shoulder height, neck perimeter and head width] and dissected. The spleen and stomach were collected and weighed separately. The stomach was opened and all of its contents were washed over a sieve (1 mm mesh), and weighed (Cavallini & Volpi, 1996). The components were macroscopically separated in nine different categories: mammals, birds, reptiles, amphibians, fish, invertebrates, plant material, eggs and carrion (Cavallini & Volpi, 1996; Rosalino *et al*., 2009), excluding the bait used in traps (eg. thighs or plucked chicken wings) (Azevedo *et al*., 2006). Each undigested component was macroscopically or microscopically analyzed, and the corresponding species or the closest taxon was identified through keys, guides, reference collections (Landry & Van Kruiningen, 1979; Cavallini & Volpi, 1996; Rosalino *et al*.,

2009), and whenever necessary DNA barcoding was used (Santos *et al*., 2015). The minimum number of individuals per stomach was estimated through the number of heads, teeth, tails, legs, and the number of fruits by the amount of seeds or peels. The components of each category were weighed and placed in the oven to dry at 77° C, for 24 hours (Lewis *et al*., 2010). After drying, each component was weighed again to the nearest scale 0.0001g (Santos *et al*., 2007).

Indices of visceral and subcutaneous fat, on a scale from 0 to 4 (method adapted from Braun, 2005) were assigned to each mongoose, wherein the amount of fat index increases proportionally with the increase of the thickness of each of these two types of fat (Bandeira *et al*., *submitted*). The age of each specimen was determined by analysis of the dental development (Bandeira *et al*., 2016). Each specimen was assigned to one of four age classes: adults, over one year of age, sub-adults, between nine and twelve months, juveniles type II, between five-and-a-half and nine months, and juveniles type I, between two-and-a-half and five-and-a-half months of age (Bandeira *et al*., 2016). The date of collection determined the season assigned to each individual. Individuals collected between January and March were considered as individuals collected in winter; spring, between April and June; summer, between July and September; and autumn, between October and December.

4.4.3. *STATISTICAL PROCEDURES*

All variables were tested for normality with Kolmogorov-Smirnov Test (with correction of Lilliefors for the significance level) (Zar, 1999) before they were used in statistical tests.

The spleen weight of each specimen is presented by a standard value in grams per 100g of animal total weight (Corbin *et al*., 2008). The body size was calculated by combining the total weight of the animal with six external physical measures, through a principal component analysis (PCA), which allows obtaining a single estimate of the body size on the first component, based on the covariance matrix of the various measures. The variables were kept in the new PCA factor, called body size, when loadings were higher than 0.70. The body condition of each individual was calculated by a regression of total body mass over the total body length, whose standard residue was assumed as the value of physical

condition (eg. Schulte-Hostedde *et al*., 2001; Schulte-Hostedde *et al*., 2005; Stevenson & Woods, 2006), where positive values refer to good body condition and negative values to poor body condition (Blackwell, 2002). The relative frequency of occurrence of each food category in each stomach (in percentage) was calculated by the number of food items of each category over the total number of items, multiplied by 100 (Barrientos & Virgós, 2006). The percentage of consumed biomass in each stomach was calculated from the dry weight of the consumed item over the total consumed biomass by dry weight, multiplied by 100 (Barrientos & Virgós, 2006). A *t* test to detect statistical differences between game species and other species within the biomass consumed was performed (Zar, 1999).

Regarding the study of diet percentages of consumed biomass, the four most represented categories were used: mammals, reptiles, amphibians and invertebrates. The effects of age, gender, region and season in the diet were analyzed using Generalized Linear Mixed Models (GLMM), where the province was used as random factor to control for the non-independence of samples from the same area. In the analyzes of each food item category, the following continuous independent variables were used: subcutaneous and visceral fat, Egyptian mongoose, European rabbit and Red-legged partridge abundances, land use, altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, NDVI, spleen weight, body condition and body size. Models created to explain the variation of each food item category included the discrete variables gender, age, region and season and the interactions region x season, region x gender, region x age, season x gender, season x age and gender x age, as well as the continuous variables NDVI, spleen weight, body size and body condition, besides other continuous variables that have presented significant differences ($p<0.05$). The selection of models using Akaike Information Criterion (AICc) was performed following the procedures of Zuur *et al*. (2009). In summary, there was a ranking of all possible models using AICc (Burnham & Anderson, 2002), and only the models with differences in the values of ΔAICc (i^{th} model - minimum AICc value) lower than 2 were considered as explanatory. When multiple models were selected or equally plausible, we used an averaging modeling approach (Burnham & Anderson 2002) with a calculation of Akaike weigths for each supported model. The relative importance of the variables was also estimated. All statistical analyzes were performed through R software (version 2.13.2), using the *lme4* (Bates *et al*., 2014) and *MuMIn* packages (Barton & Barton, 2015).

4.5. RESULTS

4.5.1. FOOD CATEGORIES AND OVERALL DIET CONTENTS

Sampling yielded a total of 122 stomachs of Egyptian mongoose with valid contents, of which 72 were from females and 50 males; 26 were from the north of Tagus River and 96 from south (Figure 4.1, Table 4.1). In terms of age distribution, 60 stomachs were grouped in adult class, 24 in sub-adult, 21 in juvenile type II and 17 in juvenile type I (Table 4.1).

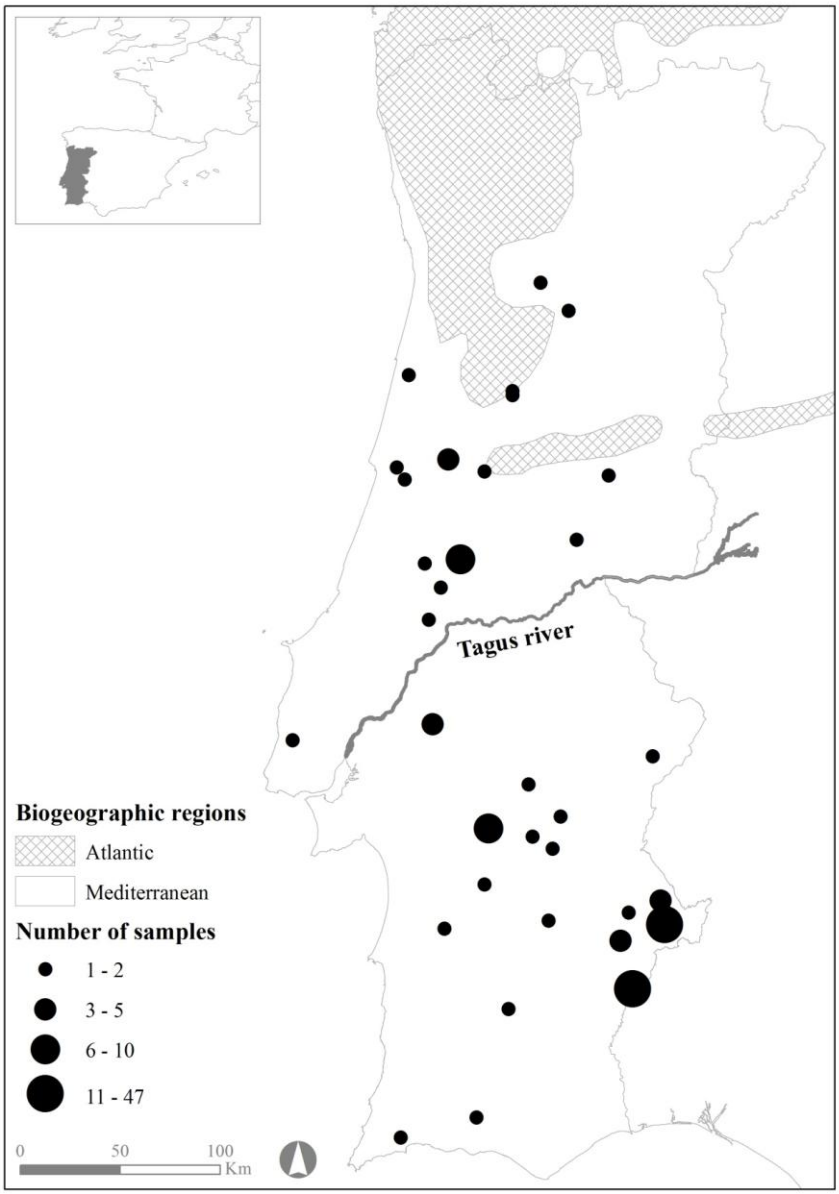

Figure 4.1. Locations of the Egyptian mongoose specimens under study.

Table 4.1. Number of samples of Egyptian mongoose obtained from each region, north and south of Tagus River, within age class and gender information.

Age Class	Gender	Region	
		North	South
Adult	Female	9	30
	Male	4	17
Sub-adult	Female	4	12
	Male	5	3
Juvenile II	Female	1	6
	Male	0	14
Juvenile I	Female	2	8
	Male	1	6
Total	Female	16	56
	Male	10	40

The body size variable was extracted from PCA which included five variables [four biometrics (snout-tail length, shoulder height, neck perimeter and head width) and total body weight] for each specimen and explained 75.1% of data variance. The body condition variable assumed the values of normalized residuals from a regression of total body weight against the total body length [$F(1,119)= 351.3741$, $p<0.001$].

Food items that occurred more frequently in Egyptian mongoose stomachs were mammals (68.85%), followed by invertebrates (33.61%), reptiles (20.49%), eggs (8.20%), amphibians (7.38%), plant material (6.56%), birds (2.46%), fish (2.46%) and carrion (1.64%) (Table 4.2). In terms of consumed biomass, there was a greater intake of mammals (63.06%), reptiles (16.27%), amphibians (7.86%), invertebrates (6.62%), carrion (4.09%), fish (1.00%), plant material (0.66%), eggs (0.34%) and birds (0.09%) (Table 4.2, Figure

4.2). Game species were represented by 40.94% of the biomass consumed (Figure 4.3) and significant statistical differences between game-related and the remainder biomass were detected by the *t* test (t = -2,979, p<0.05).

During the analysis of food components, eight mammalian, eleven reptile and four amphibian species were identified, while thirteen stomachs had composed clusters of invertebrates (Table 4.2). The Egyptian mongoose presented a diversity of species in its stomach contents, emphasizing the consumption of European rabbit, long-tailed field mouse (*Apodemus sylvaticus*) and Western Mediterranean mouse (*Mus spretus*), in the mammalian class; ladder snake (*Rhinechis scalaris*), Algerian psammodromus (*Psammodromus algirus*) and Western three-toed skink (*Chalcides striatus*), in the class of reptiles; common toad (*Bufo bufo*), in the class of amphibians; and agglomerates of dozens of invertebrates, essentially composed of grasshoppers, dragonflies, insects and beetles, although it is also notable scolopendras and crayfish consumptions (Table 4.2).

Table 4.2. Diet composition of the Egyptian mongoose in Portugal. (NS - number of stomachs where each food item was found; MNI - minimum number of individuals of each food item; RFO - percentage of relative frequency of occurrence; B - percentage of biomass consumed; NI – not identified; * - game species).

Food item	NS	MNI	RFO (%)	B (%)
Total Mammalia	**84**	**107**	**68.85**	**63.06**
Class Mammalia NI	10	10	8.20	3.55
Family Leporidae NI *	19	19	15.57	14.25
***Lepus granatensis* ***	2	2	1.64	3.90
***Oryctolagus cuniculus* ***	28	30	22.95	27.82
Order Eulipotyphla/Rodentia NI	20	21	16.39	4.58
Crocidura russula	1	1	0.82	0.43
***Arvicola* sp.**	1	1	0.82	0.19

***Microtus* sp.**	1	2	0.82	0.58
Eliomys quercinus	2	2	1.64	1.26
Apodemus sylvaticus	8	11	6.56	3.11
Mus spretus	4	4	3.28	0.74
***Rattus* sp.**	2	2	1.64	1.08
Rattus norvegicus	1	1	0.82	0.54
Rattus rattus	1	1	0.82	1.02
Total Aves *	**3**	**3**	**2.46**	**0.09**
Aves NI *	3	3	2.46	0.09
Total Reptilia	**25**	**55**	**20.49**	**16.27**
Order Squamata NI	3	3	2.46	1.32
Family Colubridae/Natricidae/ Psammophiidae/Viperidae NI	9	11	7.38	1.72
Blanus cinereus	1	1	0.82	0.07
Anguis fragilis	2	2	1.64	0.41
Coronella girondica	1	1	0.82	0.27
Hemorrhois hippocrepis	1	1	0.82	0.12
Rhinechis scalaris	4	4	3.28	6.62
Podarcis hispanicus	1	1	0.82	0.03
***Psammodromus* sp.**	4	4	3.28	0.06
Psammodromus algirus	6	10	4.92	1.57
Timon lepidus	3	3	2.46	1.02
***Natrix* sp.**	1	1	0.82	0.03
Natrix maura	1	1	0.82	0.43
Malpolon monspessulanus	2	3	1.64	0.87
Chalcides striatus	4	9	3.28	1.73
Total Amphibia	**9**	**16**	**7.38**	**7.86**

Amphibia NI	5	6	4.10	0.94
Discoglossus galganoi	1	2	0.82	0.26
Bufo bufo	5	5	4.10	6.55
Epidalea calamita	2	2	1.64	0.07
Hyla arborea	1	1	0.82	0.04
Total Actinopterygii	**3**	**3**	**2.46**	**1.00**
Actinopterygii NI	3	3	2.46	1.00
Total Invertebrates	**41**	**57**	**33.61**	**6.62**
Cluster of invertebrates NI	13	13	10.66	5.66
Class Chilopoda	3	3	2.46	0.06
Class Gastropoda NI	1	2	0.82	0.01
Class Insecta NI	6	6	4.92	0.04
Class Malacostraca NI	9	9	7.38	0.35
Order Coleoptera	7	8	5.74	0.08
Order Mantodea	1	1	0.82	0.02
Order Odonata	5	7	4.10	0.15
Order Orthoptera	8	8	6.56	0.26
Total Vegetal	**8**	**56**	**6.56**	**0.66**
Fruits NI	1	1	0.82	0.01
Grapes	2	5	1.64	0.05
Pears	1	1	0.82	0.39
Seeds	7	42	5.74	0.10
Plants NI	5	7	4.10	0.11
Total Eggs *	**10**	**14**	**8.20**	**0.34**
Total Carrion	**2**	**2**	**1.64**	**4.09**

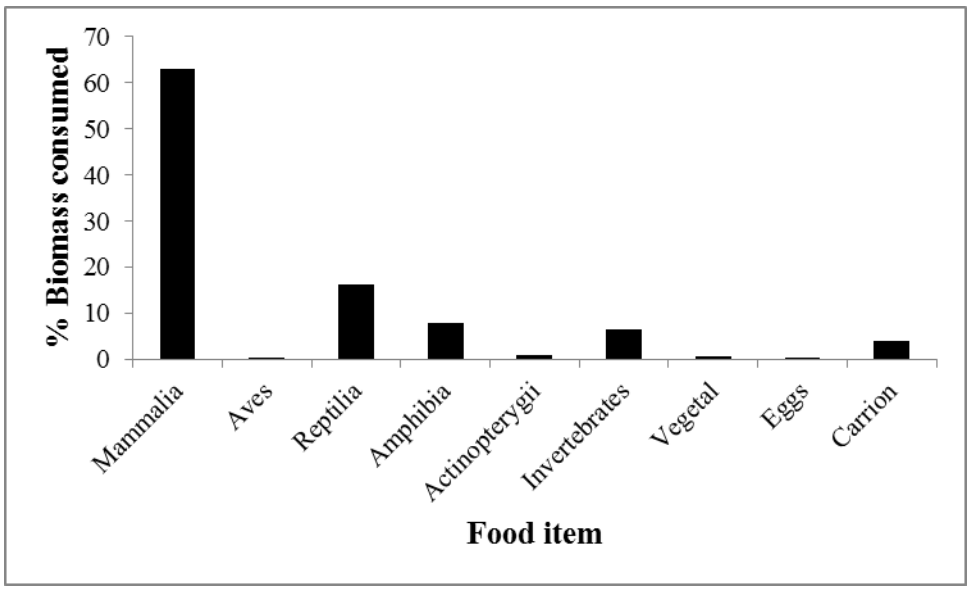

Figure 4.2. Percentage of each food item consumed by the Egyptian mongoose in Portugal.

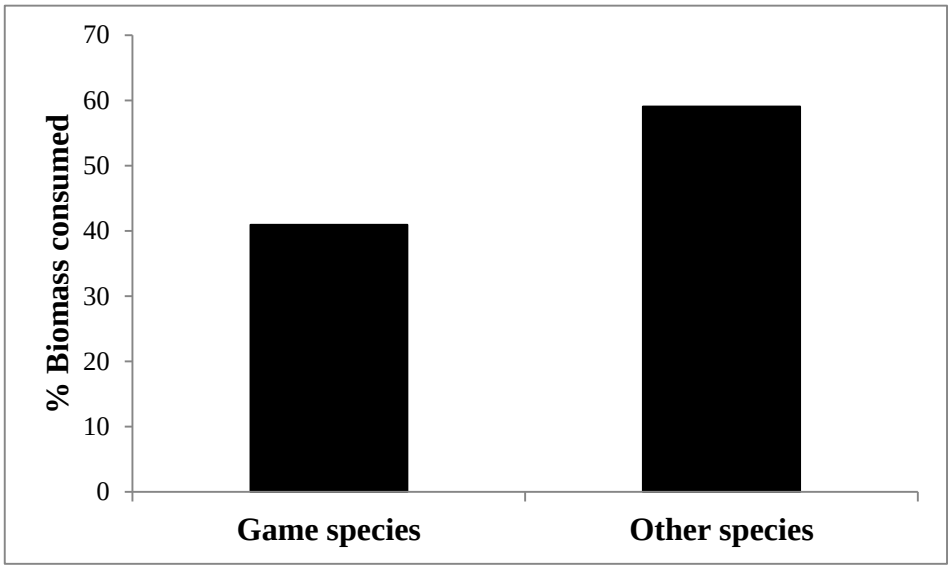

Figure 4.3. Percentage of game species and other food items consumed by the Egyptian mongoose in Portugal.

4.5.2. VARIABLES AND INTERACTIONS RELATED WITH MAMMALS' CONSUMPTION

The GLMM analysis for the mammalian intake variation in diet showed effects of province ($p<0.05$) (Table A3.1, supplementary data). After ranking all possible models using AICc, only one model with ΔAICc lower than two was obtained (Table A3.5, supplementary data). The model considered as explanatory showed the following variables: spleen weight + body condition + season + age +NDVI+ region + gender + body size + season x age + season x region + season x gender + age x region + age x gender + region x gender, with an AICc of 1005, (df = 119) (Table 4.3).

Table 4.3. Models considered as explanatory on mammal, reptile, amphibian and invertebrate food items of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	df	AICc	ΔAICc	AICc weight
Mammals				
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	1005	0.00	1.00
Reptiles				
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	929.25	0.00	0.63
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	930.29	1.04	0.37

Amphibians				
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	915.48	0.00	0.44
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	916.01	0.53	0.33
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	916.75	1.27	0.23
Invertebrates				
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	959.70	0.00	0.50
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	959.72	0.02	0.50

The diet of animals from the southern region exhibited the larger amount of mammals (Table 4.4). Regarding season, it was in the spring that higher consumption of mammals occurred, followed by autumn, summer, and less during the winter (Table 4.4). Males consumed more mammals (Table 4.4). Regarding the age class, sub-adult was the class that ingested most mammals, followed by adults, juveniles type II and juvenile type I who consumed less (Table 4.4). The animals with larger body size, good physical condition, with greater spleen weight and in areas with higher primary productivity ingested more mammals (Table 4.4). Given the estimates of the interaction between region and season, it

was found that the animals of south of the Tagus River consumed more mammals during the winter and less during summer (Table 4.4). Likewise, the estimates of interaction between region and gender revealed that males from south consumed less mammals (Table 4.4). Regarding the interaction between region and age, it was verified that southern juveniles type I consumed more mammals, while sub-adults from the same region consumed less (Table 4.4). The study of the interaction between season and gender revealed that males consumed more mammals in autumn and less in the summer (Table 4.4). The interaction between age and season verified that juveniles type I and type II, as well as sub-adults, consumed more mammals during the summer (Table 4.4). As for the gender and age interaction, it was verified that male juveniles type I consumed more mammals, followed by juvenile type II, sub-adult and adult, respectively (Table 4.4).

Table 4.4. Effects of the model and variables considered as explanatory for mammal food item of the Egyptian mongoose in Portugal.

Variables		**Estimate**	**Std. Error**	**t value**
Intercept		36.893	43.820	0.842
REGION	South	17.204	27.300	0.630
	Spring	11.545	26.291	0.439
SEASON	Summer	-10.442	34.422	-0.303
	Winter	-21.403	32.556	-0.657
GENDER	Male	16.111	26.701	0.603
	Juvenile 1	-66.163	53.108	-1.246
AGE	Juvenile 2	-15.920	39.493	-0.403
	Sub-adult	22.402	29.856	0.750
BODY CONDITION		8.988	5.036	1.785
SPLEEN WEIGHT		6.148	30.785	0.200
NDVI		12.242	60.126	0.204

BODY SIZE		3.665	9.178	0.399
	South x Spring	6.554	27.152	0.241
REGION x SEASON	South x Summer	-31.471	33.723	-0.933
	South x Winter	39.851	31.395	1.269
REGION x GENDER	South x Male	-17.271	22.455	-0.769
	South x Juvenile1	19.853	34.820	0.570
REGION x AGE	South x Juvenile2	6.776	40.064	0.169
	South x Sub-adult	-33.183	25.022	-1.326
	Spring x Male	-17.204	24.893	-0.691
SEASON x GENDER	Summer x Male	-29.435	24.574	-1.198
	Winter x Male	-21.993	27.541	-0.799
	Spring x Juvenile1	6.534	39.841	0.164
	Summer x Juvenile1	72.274	35.500	2.036
	Winter x Juvenile1	-55.873	56.593	-0.987
	Spring x Juvenile2	-77.758	38.109	-2.040
SEASON x AGE	Summer x Juvenile2	36.413	27.910	1.305
	Winter x Juvenile2	-46.778	53.733	-0.871
	Spring x Sub-adult	-2.914	31.411	-0.093
	Summer x Sub-adult	72.557	49.828	1.456
	Winter x Sub-adult	-7.150	28.242	-0.253
	Male x Juvenile1	47.357	27.484	1.723
GENDER x AGE	Male x Juvenile2	18.489	24.849	0.744
	Male x Sub-adult	15.808	29.420	0.537

4.5.3. VARIABLES AND INTERACTIONS RELATED WITH REPTILES' CONSUMPTION

The GLMM analysis for the reptile intake variation in diet showed an effect of land use (Rice fields) ($p<0.05$) (Table A3.2, supplementary data). After ranking all possible models using AICc, two models with ΔAICc lower than two were obtained (Table A3.5, supplementary data). The first model had an AICc of 929.25, (df = 37) and the second model an AICc of 930.29, (df = 36) (Table 4.3). Spleen weight, season, age, NDVI, region, gender and body size were the variables, and season x age, season x region, season x gender, age x region, age x gender and region x gender were the interactions that presented higher relative importance (Table A3.5, supplementary data).

The animals with higher spleen weight, larger body size and poor physical condition ingested more reptiles (Table 4.5). Regarding season, it was in spring that the highest consumption of reptiles occurred, followed by winter, autumn, and less during the summer (Table 4.5). In terms of the age class, juvenile type I was the class that ingested most reptiles, followed by juvenile type II, adults and sub-adults were who consumed less (Table 4.5). Animals that live in areas with lower primary productivity ingested more reptiles (Table 4.5). Animals of the southern region included fewer reptiles in the diet (Table 4.5). Males consumed fewer reptiles (Table 4.5). The estimates for the interaction between season and age indicated that juveniles type I and type II consumed more reptiles during spring and sub-adults during summer (Table 4.5). Given the interaction between season and region, it was found that the animals south of the Tagus River consumed more reptiles during the summer and less during spring (Table 4.5). The estimates of the interaction between season and gender revealed that males consumed more reptiles in spring and less during winter (Table 4.5). Regarding the interaction between age and region, it was verified that southern juvenile type I consumed less reptiles, while sub-adults from the same region consumed more (Table 4.5). As for the age and gender interaction, it was verified that male sub-adult consumed more reptiles, followed by adult, juvenile type I and juvenile type II, respectively (Table 4.5). The interaction region and gender revealed that males from south consumed less reptiles (Table 4.5).

Table 4.5. Model-averaged coefficients for the effects of explanatory variables on reptile food item of the Egyptian mongoose in Portugal.

Variables		Coefficient	Std. Error	Z value	Relative importance
Intercept		12.263	22.063	0.556	
SPLEEN WEIGHT		7.670	16.275	0.471	1.00
BODY CONDITION		-3.197	2.660	1.202	0.63
	Spring	28.363	13.613	2.084	
SEASON	Summer	-16.420	17.624	0.932	1.00
	Winter	2.149	17.206	0.125	
	Juvenile 1	52.926	28.051	1.887	
AGE	Juvenile 2	39.516	19.973	1.978	1.00
	Sub-adult	-20.905	15.706	1.331	
NDVI		-6.072	29.631	0.205	1.00
REGION	South	-5.130	13.111	0.391	1.00
GENDER	Male	-2.843	13.943	0.204	1.00
BODY SIZE		7.291	4.813	1.515	1.00
	Spring x Juvenile1	23.701	21.144	1.121	
	Summer x Juvenile1	-10.502	18.845	0.557	
	Winter x Juvenile1	14.243	29.858	0.477	
	Spring x Juvenile2	50.363	20.184	2.495	
SEASON x AGE	Summer x Juvenile2	1.011	14.784	0.068	1.00
	Winter x Juvenile2	27.275	28.465	0.958	
	Spring x Sub-adult	-13.489	16.315	0.827	
	Summer x Sub-adult	12.467	25.782	0.484	
	Winter x Sub-adult	-7.610	14.750	0.516	
	Spring x South	-34.217	14.195	2.411	
SEASON x REGION	Summer x South	18.546	17.307	1.072	1.00

	Winter x South	-6.149	16.578	0.371	
	Spring x Male	2.504	13.197	0.190	
SEASON x GENDER	Summer x Male	-1.246	12.945	0.096	1.00
	Winter x Male	-8.045	14.438	0.557	
	Juvenile1 x South	-35.856	18.421	1.946	
AGE x REGION	Juvenile2 x South	-33.767	20.239	1.668	1.00
	Sub-adult x South	26.355	13.243	1.990	
	Juvenile1 x Male	-5.982	14.565	0.411	
AGE x GENDER	Juvenile2 x Male	-10.111	13.169	0.768	1.00
	Sub-adult x Male	25.443	15.304	1.662	
REGION x GENDER	South x Male	-4.939	11.663	0.423	1.00

4.5.4. VARIABLES AND INTERACTIONS RELATED WITH AMPHIBIANS' CONSUMPTION

The GLMM analysis for the amphibians intake variation in diet didn't show any direct effect of the variables tested (Table A3.3, supplementary data). After ranking all possible models using AICc, three models with ΔAICc lower than two were obtained (Table A3.5, supplementary data). The first model had an AICc of 915.48, (df = 36), the second model an AICc of 916.01, (df = 37), and the third model presented an AICc of 916.75, (df = 36) (Table 4.3). Spleen weight, season, age, NDVI, region and gender were the variables, and season x age, season x region, season x gender, age x region, age x gender and region x gender were the interactions that presented higher relative importance (Table A3.5, supplementary data).

Animals that had lower spleen weight ingested more amphibians (Table 4.6). Regarding season, it was in the summer that the highest consumption of amphibians occurred, followed by spring, autumn, and less during the winter (Table 4.6). In terms of age class, sub-adult was the class that ingested most amphibians, followed by juvenile type I, juvenile type II, and adult were whom consumed less (Table 4.6). Animals that lived in

areas with higher primary productivity ingested more amphibians (Table 4.6). Animals of the southern region included more amphibians in the diet (Table 4.6). Males consumed more amphibians (Table 4.6). Animals with larger body size and body condition ingested more amphibians (Table 4.6). The interaction between season and age verified that juveniles type I and sub-adult consumed more amphibians during autumn and juvenile type II during spring (Table 4.6). Given the interaction between season and region, it was found that the animals of south of the Tagus River consumed more amphibians during summer and less during spring (Table 4.6). The interaction between season and gender revealed that males consumed more amphibians in winter and less during summer (Table 4.6). Regarding the interaction age and region, it was verified that the southern juvenile type II consumed less amphibians, while sub-adults from the same region consumed more (Table 4.6). As for the age and gender interaction, it was verified that were the male juvenile type II that consumed more amphibians, followed by juvenile type I, adult and sub-adult, respectively (Table 4.6). The interaction region and gender revealed that males from south consumed less amphibians (Table 4.6).

Table 4.6. Model-averaged coefficients for the effects of explanatory variables on amphibian food item of the Egyptian mongoose in Portugal.

Variables		**Coefficient**	**Std. Error**	**Z value**	**Relative importance**
Intercept		-4.480	20.046	0.223	
SPLEEN WEIGHT		-14.977	14.976	1.000	1.00
	Spring	3.499	12.441	0.281	
SEASON	Summer	11.932	15.950	0.748	1.00
	Winter	-8.851	15.843	0.559	
	Juvenile 1	8.508	25.269	0.337	
AGE	Juvenile 2	7.603	18.354	0.414	1.00
	Sub-adult	10.725	14.323	0.749	
NDVI		9.401	27.067	0.347	1.00
REGION	South	4.742	12.035	0.394	1.00

GENDER	Male	6.579	12.797	0.514	1.00
BODY SIZE		1.600	4.417	0.362	0.77
	Spring x Juvenile1	-3.676	19.324	0.190	
	Summer x Juvenile1	-9.292	17.277	0.538	
	Winter x Juvenile1	-28.314	27.462	1.031	
	Spring x Juvenile2	3.597	18.461	0.195	
SEASON x AGE	Summer x Juvenile2	-13.849	13.493	1.026	1.00
	Winter x Juvenile2	-29.533	26.053	1.134	
	Spring x Sub-adult	-10.145	14.916	0.680	
	Summer x Sub-adult	-34.642	23.715	1.461	
	Winter x Sub-adult	-0.229	13.544	0.017	
	Spring x South	-1.753	13.070	0.134	
SEASON x REGION	Summer x South	15.961	15.817	1.009	1.00
	Winter x South	14.790	15.257	0.969	
	Spring x Male	-2.588	12.135	0.213	
SEASON x GENDER	Summer x Male	-18.783	11.872	1.582	1.00
	Winter x Male	17.897	13.291	1.347	
	Juvenile1 x South	-4.009	16.841	0.238	
AGE x REGION	Juvenile2 x South	-9.810	18.469	0.531	1.00
	Sub-adult x South	0.623	12.194	0.051	
	Juvenile1 x Male	3.261	13.318	0.245	
AGE x GENDER	Juvenile2 x Male	7.912	12.121	0.653	1.00
	Sub-adult x Male	-18.033	14.027	1.286	
REGION x GENDER	South x Male	-6.845	10.736	0.638	1.00
BODY CONDITION		0.486	2.449	0.199	0.56

4.5.5. VARIABLES AND INTERACTIONS RELATED WITH INVERTEBRATES' CONSUMPTION

The GLMM analysis for the variation of invertebrates intake in diet showed effects of altimetry ($p<0.05$) and average annual temperature ($p<0.05$) (Table A3.4, supplementary data). After ranking all possible models using AICc, two models with ΔAICc lower than two were obtained (Table A3.5, supplementary data). The first model had an AICc of 959.70, (df = 36) and the second model an AICc of 959.72, (df = 37) (Table 4.3). Spleen weight, season, age, NDVI, region, gender and body size were the variables and season x age, season x region, season x gender, age x region, age x gender and region x gender were the interactions that presented higher relative importance (Table A3.5, supplementary data).

Animals with lower spleen weight and smaller body size, and those with highest body condition ingested more invertebrates (Table 4.7). Regarding season, it was in the autumn that the highest consumption of invertebrates occurred, followed by winter, spring, and less during the summer (Table 4.7). In terms of age class, adult was the class that ingested most invertebrates, followed by juvenile type I, juvenile type II and sub-adult were who consumed less (Table 4.7). Animals that lived in areas with higher primary productivity ingested more invertebrates (Table 4.7). Animals of the southern region included more invertebrates in their diet (Table 4.7). Males consumed fewer invertebrates (Table 4.7). The interaction between season and age verified that juveniles type I consumed more invertebrates during summer, while juvenile type II and sub-adult during winter (Table 4.7). Given the interaction between season and region, it was found that the animals of south of the Tagus River consumed more invertebrates during summer and less during winter (Table 4.7). The interaction between season and gender revealed that males consumed more invertebrates in summer and less during autumn (Table 4.7). Regarding the interaction between age and region, it was verified that the southern juvenile type I consumed less invertebrates, while sub-adults from the same region consumed more (Table 4.7). As for the age and gender interaction, it was verified that the male juvenile type II consumed more invertebrates, followed by adult, sub-adult and juvenile type I, respectively (Table 4.7). The interaction region and gender revealed that males from south consumed more invertebrates (Table 4.7).

Table 4.7. Model-averaged coefficients for the effects of explanatory variables on invertebrate food item of the Egyptian mongoose in Portugal.

Variables		Coefficient	Std. Error	Z value	Relative importance
Intercept		24.357	26.637	0.914	
SPLEEN WEIGHT		-23.302	19.331	1.205	1.00
BODY CONDITION		0.619	3.171	0.195	0.50
	Spring	-18.191	16.207	1.122	
SEASON	Summer	-37.702	20.866	1.807	1.00
	Winter	-4.884	20.439	0.239	
	Juvenile 1	-3.244	33.318	0.097	
AGE	Juvenile 2	-3.283	24.311	0.135	1.00
	Sub-adult	-15.921	18.605	0.856	
NDVI		18.880	36.564	0.516	1.00
REGION	South	0.357	16.313	0.022	1.00
GENDER	Male	-13.875	16.670	0.832	1.00
BODY SIZE		-11.096	5.722	1.939	1.00
	Spring x Juvenile1	3.916	25.019	0.157	
	Summer x Juvenile1	10.608	22.302	0.476	
	Winter x Juvenile1	6.791	35.525	0.191	
	Spring x Juvenile2	17.908	23.780	0.753	
SEASON x AGE	Summer x Juvenile2	-9.189	17.357	0.529	1.00
	Winter x Juvenile2	62.485	33.642	1.857	
	Spring x Sub-adult	0.238	19.473	0.012	
	Summer x Sub-adult	-16.784	31.057	0.540	
	Winter x Sub-adult	2.438	17.597	0.139	
SEASON x REGION	Spring x South	10.230	16.959	0.603	1.00

	Summer x South	34.529	20.707	1.668	
	Winter x South	-12.848	19.700	0.652	
	Spring x Male	6.563	15.644	0.420	
SEASON x GENDER	Summer x Male	47.126	15.376	3.065	1.00
	Winter x Male	19.391	17.234	1.125	
	Juvenile1 x South	-9.475	21.695	0.437	
AGE x REGION	Juvenile2 x South	-7.787	24.486	0.318	1.00
	Sub-adult x South	13.934	15.721	0.886	
	Juvenile1 x Male	-35.447	17.253	2.055	
AGE x GENDER	Juvenile2 x Male	2.065	15.617	0.132	1.00
	Sub-adult x Male	-10.086	18.287	0.552	
REGION x GENDER	South x Male	10.645	13.988	0.761	1.00

4.6. DISCUSSION

Our results confirmed the significant variation of Egyptian mongoose' diet between genders, which also had been presumed in a previous study (see Rosalino *et al*., 2009). The results confirmed that mammals and amphibians are significantly more consumed by males. An earlier study had pointed out this trend, although not significantly for amphibians (see Rosalino *et al*., 2009). Females invested in the consumption of reptiles and invertebrates. This variation may be the result of sexual dimorphism in this species caused by sexual selection (Bandeira *et al*., 2016), in which males capture prey that allow them to obtain more energy to offset the energy costs of defending and patrolling the territory and maintain a large body size and competitive interactions (Erlinge, 1981; Palomares, 1994; Thom *et al*., 2004; Zalewski, 2007; Rosalino *et al*., 2009). Variation in the exploratory behavior between individuals of different genders, due to a focus on different types and sizes of prey, would result in a reduction of intraspecific competition for resources (Zalewski, 2007; Rosalino *et al*., 2009). As males are larger than females,

they may be more apt to capture larger prey (Bandeira *et al*., 2016) as mammals. As with the Egyptian mongoose, stoat (*Mustela erminea*) males also focus on more energetic prey (Erlinge, 1981). The diet variation between genders is also described in red fox (*Vulpes vulpes*) from the Mediterranean area, wherein females eat more mammals and invertebrates than males (Cavallini & Volpi, 1996), in opposite to Egyptian mongoose. Indeed, females can consume more energetic prey to cope with large reproductive costs. However, such characteristics are not found in the diet of the Egyptian mongoose, where diet differences between genders appears to be more related with body-size and energetic constraints.

Considering age classes, it was found that older animals (sub-adults and adults) consumed more mammals, while the youngest (juveniles type II and I) invested in reptiles. As with sexual dimorphism, the dimorphism between age classes could explain this difference between ages consumption, since older animals, which on average are larger than the youngest (Bandeira *et al*., 2016), would display greater ability to capture bigger and more energetic prey as mammals, and probably only older individuals have access to best food habitats, whereas juvenile are despotically displaced to less productive habitats where they only found less energetic prey. The juvenile type I and type II may cover energy expenditure during the growth phase, investing in smaller prey and in prey whose capture or manipulation involve less energy expenditure, while improving their hunting techniques as they get older (Ben-Yaacov & Yom-Tov, 1983). In addition, considering that juvenile type I are, in part, still dependent on prey captured by the progenitor (Ben-Yaacov & Yom-Tov, 1983) and that females consume more reptiles, it is expectable that the consumption of these prey is greater at this stage. Another reason that can justify the prey consumed by juveniles is due to the state of the development of dentition. Animals of juvenile stages are in the dentition replacement phase (Bandeira *et al*., 2016), and may have more constraints in bite force (Binder & Van Valkenburgh, 2000), and so would be less able to capture more robust prey. In the red fox (*Vulpes vulpes*) no variation was found in diet between age classes (Cavallini & Volpi, 1996), with only a higher intake of invertebrate by adults, compared to juveniles, in the case of a population of Alpine red fox (Balestrieri *et al*., 2011).

The results showed that the Egyptian mongoose with larger body size consumed more vertebrates and less invertebrates. The body size of the Egyptian mongoose seems to

influence the choice of the prey to capture. Smaller body size mongooses may preferentially target the less energetic prey (invertebrates), which would not require too much strength, or technique, or improved manipulation during capture (Ben-Yaacov & Yom-Tov, 1983), and smaller mongooses can attain their energetic requirements from smaller and easily captured prey. While the vertebrate prey, more energetic and that require improved hunting techniques (Ben-Yaacov & Yom-Tov, 1983) are the preferred target of the larger size Egyptian mongoose. Again, in contrast with mongoose, body size in red fox is not related to consumption of any food item (Cavallini & Volpi, 1996).

The results showed that the mongoose with heavier spleens consumed higher amounts of mammals and reptiles. This relationship may be explained by the fact that these items are the most consumed by males and females, respectively, where this peak consumption coincides with the breeding season of the winter and spring, induced by the reproductive behavior of both genders (Bandeira *et al*., *submitted*). It is especially during this period that the Egyptian mongoose engage in physical aggression in defense of the territory, to access to the largest number of females or to dispute for prey (Palomares, 1991; Palomares, 1993), and that females copulate and give birth (Palomares & Delibes, 1992), which increases the exposure to new antigens (Fernández-Llario *et al*., 2004), as it is also at this stage that there is an increase of the damage caused by aggressions of progenitor during the expulsion of offspring in the independence period (Palomares, 1991; Palomares & Delibes, 1998). All these factors can jointly stimulate the immune system that may be reflected by an increase in spleen weight as a response to immunity challenges (Bandeira *et al*., *submitted*). Furthermore, if spleen size can be a surrogate of individual quality (e.g. better immunocompetence), then a link between better and healthier individuals and better hunting efficiency could be predictable. On the other hand, the supposed link could be a consequence of better diet (e.g. more energetic mammals) producing more immunocompetent individuals.

Our results also indicated that Egyptian mongoose with better body condition consumed more mammals and amphibians, and fewer reptiles and invertebrates. The most consumed items for animals with better body condition coincided with the most consumed items by males, which have better physical condition relatively to females (Bandeira *et al*., *submitted*). Putting these findings together, we thus assume that males that will consume

more mammals and amphibians will present better body condition, comparatively to females, which by consuming more reptiles and invertebrates will present poorer physical condition, confirming the suggestions set out in previous studies (see Rosalino *et al*., 2009). Thus it is suggested that the mongoose that would consume more energetic prey (such as mammals), will present a more favorable energetic level, that in the case of males benefits the reproductive behavior, due to roaming and defending large territories (Palomares, 1991), favoring those that are better prepared and in better physical condition (Marker & Dickman, 2003). Sexual dimorphism in the use of ecological niche and in the differentiation of consumed prey seems to favor the distinction in physical condition of the animals.

When an animal presents a good energy state, it is more able to invest in the production of lymphocytes and storage of red blood cells (Schute-Hostedde & Elsasser, 2011). Considering that the consumption of mammals is positively associated with heavier spleens and good physical condition, our study also suggests that animals that consume predominantly mammals may exhibit both improved body condition and immune competence.

As in Spain (Delibes *et al*., 1984; Palomares & Delibes, 1991*a*; Palomares & Delibes, 1991*b*; Palomares, 1993), a significant seasonal variation was found in the mongoose diet in Portugal. As for the differences in consumption across seasons, it was observed that mongoose ingested more mammals and reptiles during the spring, while in summer they invested in amphibians and, in invertebrates during the autumn. In winter they consumed more reptiles and invertebrates. Knowing that males consumed more mammals, and females more reptiles, a synchronism can be found here, with the fact that it is precisely during spring that the breeding season of the Egyptian mongoose occurs, wherein the defense and patrolling of the territory by males is more pronounced, requiring the intake of more energetic prey (eg. mammals), and wherein females require more energy for reproduction, spending the least in prey capture by investing in energy restraint, opting for less energetic prey (eg. reptiles) (Palomares, 1994; Rosalino *et al*., 2009), corroborating the same explanation of the results above, about the difference of consumed items between gender. Furthermore, this increase of mammals' and reptiles' consumption during spring coincides with periods of the reproductive activity of these two classes of prey: on the one

hand, it occurs within the period in which young rabbits remain in burrows, which mongoose invade (Palomares, 1993) and during which myxomatosis incidence may be greater, leading to rabbit morbidity (Palomares & Delibes, 1991*a*), favoring prey capture without their own health being affected (Rosalino & Santos-Reis, 2002; Ferreras *et al*., 2011). Secondly, it is also during this period that the beginning of the basking behavior of reptiles occurs, after a period of lower activity in winter, and of a lower mobility of pregnant females (Rosalino & Santos-Reis, 2002; Kaliontzopoulou *et al*., 2013). Thus, the mongoose finds less mobility in this prey class, which could enable more captures with less physical effort, in addition to that it is also during this time that larger quantities of juvenile reptiles can be found (Palomares & Delibes, 1991*a*; Kaliontzopoulou *et al*., 2013). A greater amphibian's consumption in the summer may be related to the fact that this is the warmest and driest period of the year when Egyptian mongoose spends more time near water points (Matos *et al*., 2009; Santos *et al*., 2011) where amphibians also seek refuge, some of which burrowing since during this period they are in lower activity. The intense exploratory activity of this predator thus facilitates uncovering amphibians (Palomares & Delibes, 1998). The consumption of invertebrates during the autumn may be related to an increased abundance of these prey items in this period; apart from that, they are easier to capture and subdue, and therefore appreciated by juvenile type II (most abundant at this season), who are initiating food dependence on their own exploratory activity (Ben-Yaacov & Yom-Tov, 1983). It is also likely that, during this period, adults have to capture more energetic prey to provide for offspring, supplementing their own diet with the capture of invertebrates. The winter period promotes lower activity in groups of reptiles and invertebrates, and ends up increasing their consumption due to the digging and exploratory habits of the Egyptian mongoose, which would benefit them (Palomares & Delibes, 1991*c*; Palomares & Delibes, 1998; Matos *et al*., 2009). In the case of sympatric species as red fox (*Vulpes vulpes*), there is also a seasonal variation in the diet, in which vertebrates also predominate during the mating and birth season, and invertebrates in summer (Cavallini & Volpi, 1996). Foxes, as Egyptian mongoose, seem to use the available resources, taking into account the abundance and energy needs it faces throughout the year. Other sympatric species alternate the consumption of the food items (Beja, 1997; Fedriani *et al*., 1998; McDonald *et al*., 2000; Rosalino & Santos-Reis, 2002; Carvalho & Gomes, 2004; Rosalino *et al*., 2005).

Diet variability between different areas and regions, found out either in Portugal (Santos *et al*., 2007; Rosalino *et al*., 2009) or in Spain (Delibes *et al*., 1984; Palomares, 1993), was also confirmed with the results of this study. We found that, in the north, the Egyptian mongoose invested more in the consumption of reptiles, while in the south it ate mostly mammals, amphibians and invertebrates. In Portugal, the composition and richness of species differs geographically (eg. Mathias *et al*., 1999; Cabral *et al*., 2005; Loureiro *et al*., 2008). In general, most game reserves are located in southern area of the Tagus River, which provides large concentrations of high quality prey, such as European rabbit and red-legged partridge (Beja *et al*., 2009). This added value in the south favors populations with established territories in these areas, as they provide food in greater quantity and quality, because many of these reserves maintain a constant management of their own animal populations and habitats (*personal observation*). This management indirectly provides excellent conditions for opportunistic predatory species like the Egyptian mongoose such as shelter, water and food. In the north, a greater lack of these game species in large concentrations requires the mongoose to adapt to habitat conditions, by focusing on available species (Delibes *et al*., 1984; Palomares & Delibes, 1998) that are more diverse in the Mediterranean area (Virgós *et al*., 1999).The results indicated that, in areas with higher NDVI, more mammals, amphibians and invertebrates, and fewer reptiles were consumed. Places with higher primary productivity offer greater opportunities for higher biomass of primary consumers, who are the mongooses' prey (Rogers & Myers, 1979; Palomares & Delibes, 1990; Palomares & Delibes, 1991*a*; Palomares & Delibes, 1993; Santos *et al*., 2011). Thus, most consumed items in higher primary productivity areas seem to be those that are most dependent on the proximity of the water to survive, such as amphibians and invertebrates; and on good plant and shrub cover for food and shelter, in the case of mammals. The reptiles, despite also being found and captured on productive zones, due to their greater mobility and independence from the presence of water, comparatively to other classes under study, become the most abundant prey group in areas with lower productivity rates (Delibes *et al*., 1984).

Heading the hunters' paradigm, this study suggests that the Egyptian mongoose does not direct its captures to game species, in contrast to what was percieved by this community. However, populations of Egyptian mongoose whose territories overlap with hunting areas and, consequently higher densities of European rabbit and red-legged partridge, may

opportunistically take advantage of this food source, but they deviate to other species when these prey species are lacking. In this work, we found that consumption of wild game species, including leporides, birds and eggs, is less than half of the total consumption of this carnivore's diet, both in frequency and in biomass. Given the differences between genders, it was found that males, which consume more mammals than females, may be the most disadvantageous gender predator for game species, such as European rabbit. Thus, it is important to understand if there are behavioral differences between genders in the exploratory patterns, given that males seem to have underground exploratory behavior, that allows them to invade burrows and dens (mammals) (Palomares, 1993), or dig up prey (amphibians), while females appear to prefer to chase prey (reptiles) and capture them on the soil surface (invertebrates). In this sense, we suggest that radio-tracking studies would complement this work, enabling to gain further insights into the behavioral ecology of Egyptian mongoose. Younger Egyptian mongoose consumed more reptiles than adults and sub-adults, and they may be useful in controlling the populations of these species, perhaps being the carnivore of native fauna which includes the highest percentage of reptiles in the diet. Thus, during the planning of correction density actions, these implications should be taken into consideration.

The expansion area of the Egyptian mongoose to the north of the Iberian Peninsula may be a threat to some species classified as endemic. Thus, it becomes imperative to promote the continuity of this carnivore's diet monitoring, while it expands its distribution, due to the imbalance that it may cause to some of these more fragile populations. Further, it is necessary to study the habitat preference of the species in the north of the Tagus River, since in the south it is already known that the Egyptian mongoose is established in the higher primary productivity levels (Santos *et al*., 2011).

Given the variations in the species diet in each season, we suggest that the Egyptian mongoose, a generalist and opportunistic predator, will take advantage of different food items, depending on availability, abundance and vulnerability, taking into account the specific energetic needs. This predator seems to take advantage of its digging and exploratory habits, combined with the different periods of less physical mobility and greater ecological activity of each taxon in order to spend as minimum energy as possible in prey capture .

In a nutshell, we confirm that the trophic ecology of the Egyptian mongoose reflects the species predatory behavior that, due to sexual dimorphism, may explore the environment in two dimensions diversifying its diet between genders, and that this species' diet footprint varies along ecological gradients in its European range, finding positive effects both from primary productivity, season and individual traits.

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CHAPTER 5

REPRODUCTION

5.1. The reproductive ecology patterns of the Egyptian mongoose (*Herpestes ichneumon*) on the western limits of Europe

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5.2. ABSTRACT

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a carnivore predator in expansion on the western limits of Europe, whose ecology and reproductive traits remain overlooked. It is unpopular within the hunting community that deems it to generate multiple large litters and to predate mostly on small game species with great economic importance. However, reproductive patterns of the species in Portugal are unknown.

To determine the periods of reproductive activity and produce baseline data for population management purposes, we weighed and measured the reproductive organs of 507 free-ranging specimens of Egyptian mongoose. These samples were obtained from hunting activities and accidental road kills, throughout all seasons and from seven provinces of mainland Portugal. The periods of lactation and gestation were determined for females (n=10), as well as the number of embryos or fetuses found in the reproductive systems of each pregnant individual (n=20), in order to obtain the average litter size. The age class, sampling season, region and primary productivity of their origin, as well as spleen weight and body condition of each specimen, were related with ovarian and testicular weights, with parameters of ecological, environmental, climatic and habitat pressures, as well as with visceral and subcutaneous fat.

Our results indicated that females have an active reproductive period between December and June, with a leading period from February onwards, and an inactive period during the rest of the year. Males appear to be reproductively active throughout the year. Considering the weights of reproductive organs, both genders showed a synchronized peak in reproductive activity during February. Females with heavier ovaries were found in spring, exhibited heavier spleens, and originated from areas with higher primary productivity (NDVI). Pregnant females were found between March and July, although there were exceptions. Lactating females were identified between March and August. Males with heavier testes were adults, exhibit poorer body condition and lower spleen weight, and are found in the northern region, during the summer and winter, and in places with lower NDVI. The litter size ranged from one to four cubs, however the most likely situation found was three cubs (modal value). These data do not support the idea that Egyptian mongoose generates multiple or numerous litters in Portugal.

Our results indicate that reproductive traits in females are mostly influenced by season. Males on the other hand, are also greatly influenced by their territorial success and by immune function in addition to seasonality. These results support the notion of a seasonal, male-specific, energetic trade-off between reproductive performance and body condition and immune function, a conflict that has also been confirmed for other top predators.

KEYWORDS

Herpestes ichneumon; Egyptian mongoose; Iberian Peninsula; reproduction; ovaries weight; testes weight; litter size.

5.3. INTRODUCTION

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a medium-sized carnivore whose geographic distribution in Europe is confined to the western part of the Iberian Peninsula (Delibes *et al*., 1984; Dobson, 1998; Cabral *et al*., 2005). It has been expanding in recent years towards the north and west (Talegón & Parody, 2009; Recio & Virgós, 2010; Barros & Fonseca, 2011; Balmori & Carbonell, 2012; Barros *et al*., 2015). In the Iberian range, the Egyptian mongoose falls under two different hunting regimens: while hunting is prohibited in Spain, it is a game species under Portuguese law ("Decreto-Lei nº 201/2005"). Hunters consider that Egyptian mongoose compete with their interests, based on the general perception that it generates multiple large litters and that the European rabbit (*Oryctolagus cuniculus*), red-legged partridge (*Alectoris rufa*) and their eggs, are at the center of its diet, both of which are fundamental for the economy of small game in the Iberian Peninsula (Virgós & Travaini, 2005). However, recent work by our team suggests the diet of this mongoose to be more diverse than previously acknowledged, with more than a half of the feeding spectrum consisting of food items that are not game species, such as reptiles, amphibians or invertebrates (Bandeira *et al*., *unpublished data*), contrary to the prediction of hunters. In fact, the diet composition and guild of prey species differ from region to region in the Portuguese territory (see Mathias *et al*., 1999; Cabral *et al*., 2005; Loureiro *et al*., 2008; Bandeira *et al*., *submitted*). The nutritional status and energy uptake is expected to influence reproductive ecology, since males need more energetic prey to defend and patrol their territory, while females consuming prey that require less energy and time to capture, may invest more on reproductive demands (Rosalino *et al*., 2009). Other recent studies on the Egyptian mongoose ecology have provided new knowledge on this species in the Portuguese territory, from biometric studies (Bandeira *et al*., 2016) body condition and immune competence (Bandeira *et al*., *submitted*). In the latter work, it is suggested that males, as well as adults compared with other age classes, have better body condition, and a positive correlation was found with spleen weight, supporting our prediction that spleen weight as proxy for immune function would be dependent on body condition, as the immune system is energetically costly (Bandeira *et al*., *submitted*). Spleens of males are heavier, peaking in specimens captured during the winter and spring (Bandeira *et al*., *submitted*). We hypothesize that spleen weight variation may be related to

gender specific behavior and hormonal variations, since the Egyptian mongoose is a polygynic species (Palomares, 1993*a*), with seminal work pointing out for different investments in reproduction and survival between genders (Palomares, 1993*a*; Palomares & Delibes, 1993*b*). However, a knowledge gap remains regarding the species' reproductive ecology. Reproductive ecology is a key element of the natural history of species, linking issues from ecological and physiological attributes and affecting one of the pivotal elements of fitness, the differential reproduction of individuals. In Portugal, the reproductive period and litter size of this species are unknown, as well as the influence exerted by factors presumed to affect reproductive traits, such as age, body condition, spleen weight, season, geographical region' conditions or primary productivity.

In southwestern Spain, heavier males exhibit a polygynous behavior and those with lower body mass appear to be monogamous (Palomares, 1993*a*). Males have active spermatogenesis and sperm throughout the year, indicating that they can reproduce at any time (Palomares, 1993*b*). Copulations take place between February and June, but more often between March and April (Palomares & Delibes, 1992; Palomares, 1993*b*), while in Israel, another region of the Mediterranean basin where the species is present, copulations take place earlier, between February and March (Ben-Yaacov & Yom-Tov, 1983). In Spain, births take place between April and August, most frequently between May and July, after a pregnancy lasting 72 to 88 days (Palomares & Delibes, 1992; Palomares, 1993*b*) and in Israel, during April and May, after a gestation period of two months (Ben-Yaacov & Yom-Tov, 1983). The average number of cubs per litter in Spain is 2.7 (n = 7) (Palomares & Delibes, 1992), and they can give birth two to four cubs (Palomares, 1993*b*). In Israel, the litters have an average of 3.3 ± 0.67 cubs (n = 10), with a range of one to four (Ben-Yaacov & Yom-Tov, 1983). Generally, they have only one litter per year, but if they lose their cubs, the breeding pair may breed again in the same year (Ben-Yaacov and Yom-Tov, 1983). Sexual maturity is reached at the age of two years (Palomares, 1993*b*).

Reproduction in the *Herpestes* gender has been studied in small Indian mongoose (*H. auropunctatus*) (eg. Soares & Hoffmann, 1981; Soares & Hoffmann, 1982; Hoffmann *et al*., 1984; Hays, 1999) and in Javan mongoose (*H. javanicus*) (Abe *et al*., 2006). The reproductive studies of small Indian mongoose introduced in several islands of the northern hemisphere (eg. Hawaii) indicates that ovarian weight decreases significantly during the

non-breeding season, between August and December, and presents the highest values between February and July, the main breeding season (Hoffmann *et al*., 1984). Even without climate variability, a well-defined breeding season remains (Hoffmann *et al*., 1984). This species exhibits different seasonal rhythms in reproductive activity (Soares & Hoffmann, 1981). The small Indian mongoose's testes are heavier during the reproductive period and less during the inactive (Soares & Hoffmann, 1981; Soares & Hoffmann, 1982). These organs experience a period of recrudescence between December and February, and are reproductively active between February and August (Soares & Hoffmann, 1981).

In carnivores, the reproductive cycle is regulated by photoperiod, and males of seasonally monoestric or polyestric species undergo a period of testicular activity and another of inactivity (Ruiz-Olmo, 1997) [although with exceptions, as in some cases of Eurasian otter's populations (*Lutra lutra*), who are sexually active throughout the year (Sidorovich & Tumanov, 1994)]. In these males testicular size is an indicator of spermatogenic activity and the beginning of sexual activity, over the course of the reproductive cycle (Cavallini & Santini, 1996; Ruiz-Olmo, 1997). In females, the beginning of reproductive activity is indicated by the increasing mass of the ovaries, which is due to foliculogenesis and later luteal activity (Carnaby *et al*., 2012).

In this study, we set out to refine our current knowledge on the reproductive traits of Egyptian mongoose. First, we determine litter sizes, the timings of pregnancy and lactation in females. Secondly, by comparing the weights of reproductive organs (ovaries and testes) obtained from a large sample of Egyptian mongoose specimens collected in an extant area of the western limits of Iberia Peninsula, we aim to understand whether and how the primary productivity of habitats, spleen weight (as a proxy of immune capacity), and body condition influence the reproductive individuality of Egyptian mongoose, in each gender, age class, season and region, under different environmental, climatic and ecological pressures. We suppose that males and females exhibit variation in the weight of their reproductive organs throughout the year that are indicative of the periods in which they are active or inactive and we predict that reproductive timings vary geographically, and are influenced by body condition and immune function. Thirdly, we test if there are gender-specific conflicts in resource allocation between mating effort or parental investment traits and immune competence.

5.4. MATERIAL AND METHODS

5.4.1. *STUDY AREA*

Wild specimens in this study were collected from 7 of 9 provinces (dimensioned based on history, common land use and related issues) in mainland Portugal, where the species is distributed.

In this study, the Tagus River was considered a geographic barrier, given that the species was more abundant in the south up to the 90s of last century (Barros & Fonseca, 2011; Barros *et al*., 2015). This barrier divides two regions, north and south, considered different due to ecological, climatic and human pressure factors (Bandeira *et al*., 2016). The south is dominated by agroforestry habitats ("montados") and shrubs, with higher temperatures and lower levels of rainfall (Bandeira *et al*., 2016). While in the north, human pressure is higher, with more roads, greater habitat variability and fragmentation (monoculture of *Eucaliptus* sp. overlap native flora, as *Quercus robur*, *Salix* sp. or *Alnus glutinosa*) (Alves *et al*., 2009; Bandeira *et al*., 2016). Specimens were assigned to one of the two regions divided by this water flow according to their origin.

Each Egyptian mongoose was assigned values of ecological and climatic variables represented as mean values for the 2 x 2 km grid where it was collected. These variables included the number of hectares allocated to each habitat type (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests and agriculture areas) (Corine Land Cover 2006). The altimetry values refer to the mean in meters per grid (data SRTM, NASA, resolution of 30 meters, ASTER Global Digital Elevation Model platform) (ASTER, 2015). The human population density values are presented as the number of inhabitants per square kilometer in each cell (data from Eurostat per kilometer) (European Commission, 2015). The road and river network values refer to the distance in meters of road (IGP, 2015) and hydrographic network (SNIRH, 2015), respectively, per grid cell. The average annual temperature and annual temperature range values allude to temperature in Celsius degrees multiplied by 10 in each cell (data from BioClim, 2015) (Hijmans *et al*., 2005). The annual rainfall values were presented in mm per cell (data from BioClim, 2015) (Hijmans *et al*., 2005). The Normalized Difference

Vegetation Index (NDVI) values, reflects means of primary productivity per cell (Pettorelli, 2013). NDVI is a ratio of reflection of spectral bands in the infrared and red in the NASA's Moderate Resolution Imaging Spectroradiometer (MODIS, 2015). Values closer to 1 are the values of NDVI with higher primary productivity and values closer to -1 are those with lower productivity (Stabach *et al*., 2015). Finally, variables representing Egyptian mongoose (*Herpestes ichneumon*), European rabbit (*Oryctolagus cuniculus*) and red-legged partridge (*Alectoris rufa*) abundances were included, based on hunting bags (ICNF, *unpublished data*).

5.4.2. *SAMPLING PROCEDURES*

A total of 678 carcasses of Egyptian mongoose were collected between January 2008 and December 2014 from hunting activities or accidental road kills. Their use was approved by national authorities. The carcasses were labeled with the date and place of collection, and frozen at -20°C. Thawed carcasses were sexed, weighed, measured (snout-tail length) and dissected (Bandeira *et al*., 2016). Lactating females were identified according to the internal development of the mammary glands and external nipples, as described elsewhere (Hoffmann *et al*., 1984; King *et al*., 1996; Abe *et al*., 2006). Spleen and reproductive organs were collected and weighed separately. The male reproductive system was dissected, with both testicles extracted from testicular sacs and separated from the epididymi (Soares & Hoffmann, 1981; Soares & Hoffmann, 1982). The two testes were weighed together and measured in length and width with a caliper (Soares & Hoffmann, 1981; Soares & Hoffmann, 1982; Parsons *et al*., 2013). Ovaries were dissected from the female reproductive tract, weighed together to the nearest 0.0001g scale and measured (Hoffmann *et al*., 1984; Parsons *et al*., 2013). The number of embryos or fetuses in the developing female uterus was counted to calculate the average litter size (Hoffmann *et al*., 1984; Abe *et al*., 2006; Parsons *et al*., 2013). Months of gestation or lactation were assigned based on the specimen's collection date. For the present study, we only used the reproductive systems of 507 animals. Individuals which could not be assigned to an age

class by dentition analysis, as well as those whose reproductive system was incomplete or damaged were rejected.

Indices of visceral and subcutaneous fat, on a scale from 0 to 4 (method adapted from Braun, 2005) were assigned to each mongoose, wherein the amount of fat index increases proportionally with the increase of the thickness of each of these two types of fat (Bandeira *et al*., *unpublished data*). The age of each specimen was determined by analysis of the dental development (Bandeira *et al*., 2016). Each specimen was assigned to one of four age classes: adults over one year of age, sub-adults between nine and twelve months, juveniles type II between five-and-a-half and nine months, and juveniles type I between two-and-a-half and five-and-a-half months of age (Bandeira *et al*., 2016). The date of collection determined the season assigned to each individual. Individuals collected between January and March were considered to be collected in winter; spring, between April and June; summer, between July and September; and autumn, between October and December.

5.4.3. *STATISTICAL PROCEDURES*

All variables were tested for normality with Kolmogorov-Smirnov Test (with correction of Lilliefors for the significance level) (Zar, 1999).

The mass of the ovaries and testes was corrected for the total weight of each animal and is presented as a standard value in milligrams per 100 g of animal total weight (Soares & Hoffmann, 1981; Hoffmann *et al*., 1984). Similarly, the spleen weight is presented as a standard value in grams per 100 g of total animal weight (Corbin *et al*., 2008). The body condition was calculated by using a linear regression of total body mass over the total body length, whose standard residues were assumed as the values of physical condition of each individual (eg. Schulte-Hostedde *et al*., 2001; Schulte-Hostedde *et al*., 2005; Stevenson & Woods, 2006), wherein positive values refer to good body condition and negative values to poor body condition (Blackwell, 2002).

The effects of age, region and season on the ovarian and testicular weights were analyzed using Generalized Linear Mixed Models (GLMM), where the province was used as a random factor to control the non-independence of the samples from the same area. In the

analyses of each type of organ, ovaries or testes, we used as continuous independent variables: subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, habitat type, altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, NDVI, spleen weight and body condition. Only *p* values lower than 0.05 were considered statistically significant.

Models created to explain the variation of ovarian and testicular weight included the discrete variables age, region and season and the interactions region x season, region x age and season x age, as well as the continuous variables NDVI, spleen weight and body condition. The selection of models using Akaike Information Criterion (AICc) was performed following the procedures of Zuur *et al* (2009). In summary, there was a ranking of all possible models using AICc (Burnham & Anderson, 2002), and only the models with differences in the values of ΔAICc (i^{th} model - minimum AICc value) lower than 2 were considered as explanatory. When multiple models were selected, we used a multi-model averaging approach (Burnham & Anderson 2002). The relative importance of the variables was also estimated.

All statistical analyses were performed using R (version 2.13.2), with the *lme4* (Bates *et al*., 2014) and *MuMIn* packages (Barton & Barton, 2015).

5.5. RESULTS

Sampling yielded a total of 507 reproductive systems of Egyptian mongoose, 269 females and 238 males, of which 137 were from the north of Tagus River and 370 from south (Figures 5.1 and 5.2, Table 5.1). In terms of age, 273 reproductive systems were grouped in the adult class, 81 in the sub-adult, 91 in the juvenile type II and 62 in juvenile type I (Table 5.1).

The body condition variable assumed the values of normalized residuals from a regression of total body weight against the total body length [$F_{females}(1,267)=419.898$, $p<0.001$; $F_{males}(1,236)=523.336$, $p<0.001$].

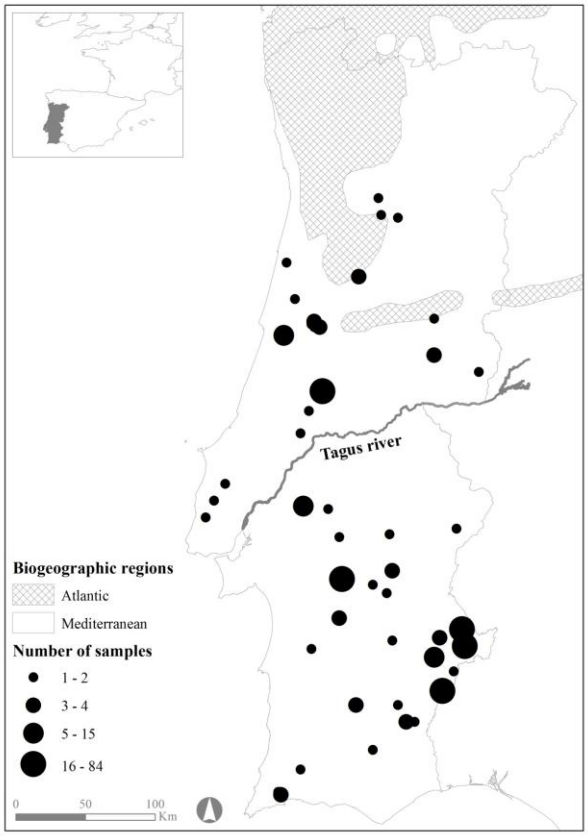

Figure 5.1. Locations and number of samples of the female Egyptian mongoose specimens under study.

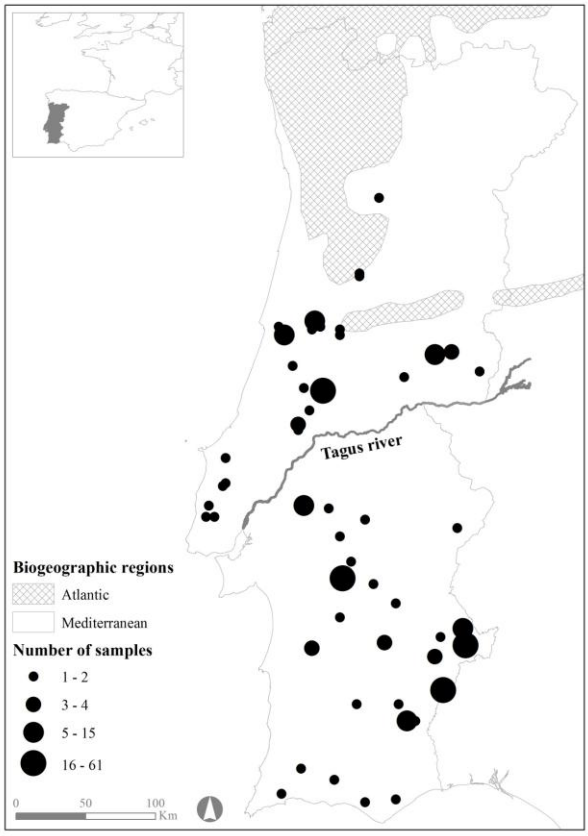

Figure 5.2. Locations and number of samples of the male Egyptian mongoose specimens under study.

Table 5.1. Number of samples of Egyptian mongoose obtained from each region, north and south of Tagus River, within age class and gender information.

Age Class	Gender	Region	
		North	South
Adult	Female	47	110
	Male	35	81
Sub-adult	Female	12	31
	Male	16	22
Juvenile II	Female	5	31
	Male	9	46
Juvenile I	Female	4	29
	Male	9	20
Total	Female	68	201
	Male	69	169

Ovarian weights of adults presented a variation throughout the year, demonstrating two different periods, one with heavier ovaries from December to June (except January, when lower values were registered), and a second period between July and November, with lower ovarian weights (Figure 5.3). This variation was confirmed by measurements of the lengths and widths of the ovaries, which increase or decrease in the same proportion and in the same periods (Figure 5.4).

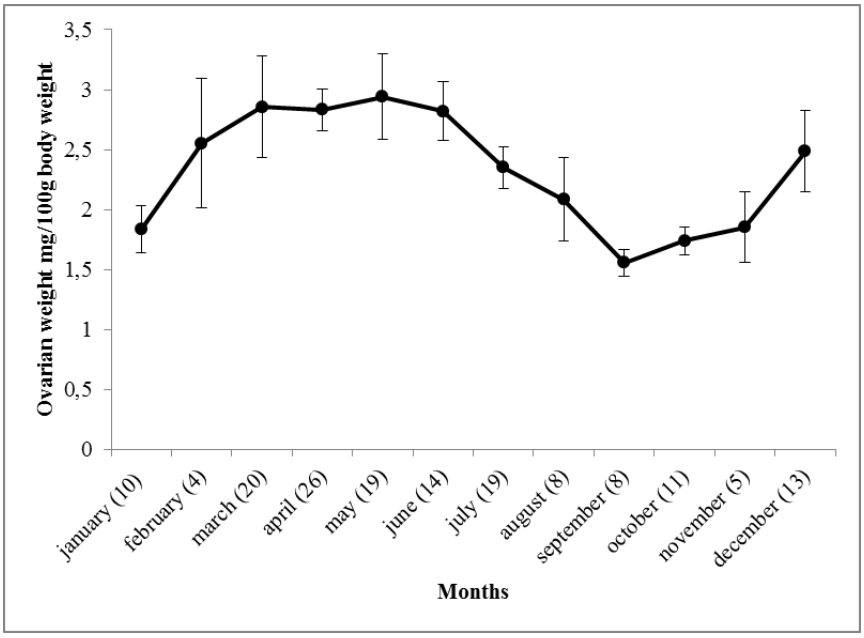

Figure 5.3. Ovarian weight (expressed as mg/100g of total body weight) of 157 adult females of the Egyptian mongoose over month. (n) is the number of females in each month. Vertical lines represent the standard error of the mean.

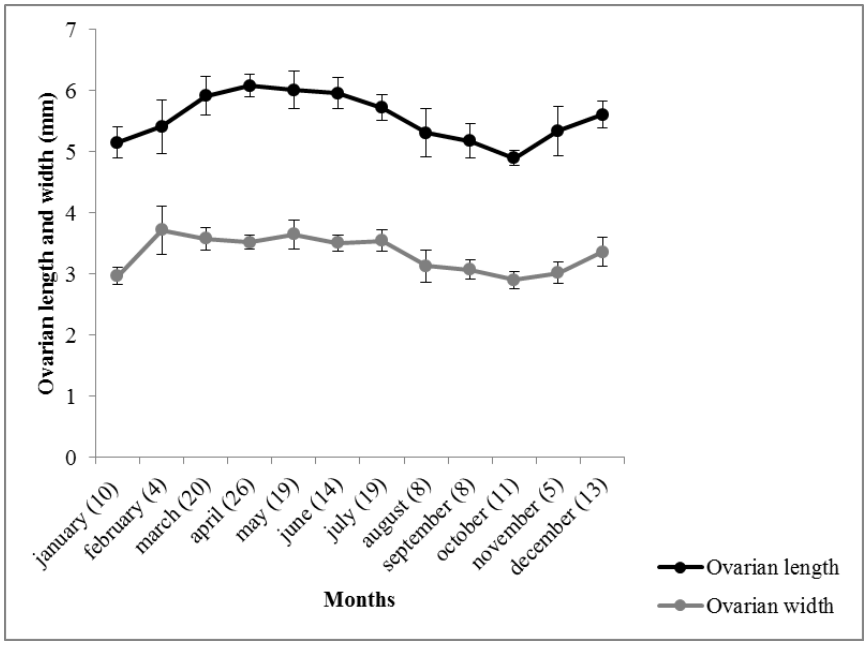

Figure 5.4. Length and width of ovarian of 157 adult females of the Egyptian mongoose over month. (n) is the number of females in each month. Vertical lines represent the standard error of the mean.

The GLMM analysis for ovarian weight variation showed effects of European rabbit (p<0.05) and red-legged partridge (p<0.05) abundances, as well as land use (rice fields) (p<0.05) (Table A4.1). After ranking all possible models using AICc (Table A4.3, supplementary data), five models with ΔAICc lower than two were obtained (Table 5.2). Season was the variable that presented higher relative importance (Table 5.3). Females with heavier ovaries were found in spring, followed by winter, summer and autumn (Table 5.3). Females with heavier ovaries were found in areas with higher NDVI (Table 5.3). Females with heavier ovaries were found in rice field areas (Table 5.3). Females with heavier ovaries exhibited heavier spleens (Table 5.3). For some details, we refer to the average data presented in Figure 5.5 and to scatterplots graphs of Figures 5.6 and 5.7.

Table 5.2. Models considered as explanatory on ovarian weight (expressed as mg/100g body weight) of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	**df**	**AICc**	**ΔAICc**	**AICc weight**
SEASON + NDVI	7	805.40	0.00	0.31
SEASON + NDVI + RICE FIELDS	8	806.12	0.72	0.22
SEASON + RICE FIELDS	7	806.37	0.98	0.19
SEASON	6	806.69	1.30	0.16
SPLEEN WEIGHT + SEASON + NDVI	8	807.23	1.83	0.12

Table 5.3. Model-averaged coefficients for the effects of explanatory variables on ovaries weight of the Egyptian mongoose in Portugal.

Variables		Coefficient	Std. Error	Z value	Relative importance
Intercept		1.997	0.388	5.140	
SEASON	Autumn	-0.249	0.208	1.198	1.00
	Spring	0.429	0.196	2.189	
	Summer	-0.125	0.214	0.582	
NDVI		0.858	0.563	1.525	0.65
RICE FIELDS		0.041	0.014	2.937	0.41
SPLEEN WEIGHT		0.154	0.435	0.354	0.12

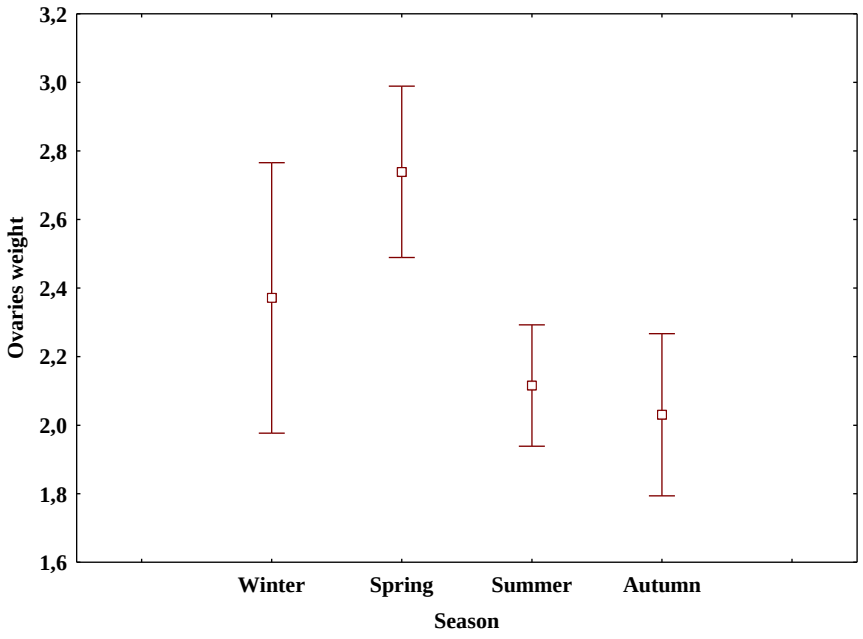

Figure 5.5. Means of 269 Egyptian mongoose ovarian weight (expressed as mg/100g of total body weight) over season. Vertical bars denote 95% confidence intervals.

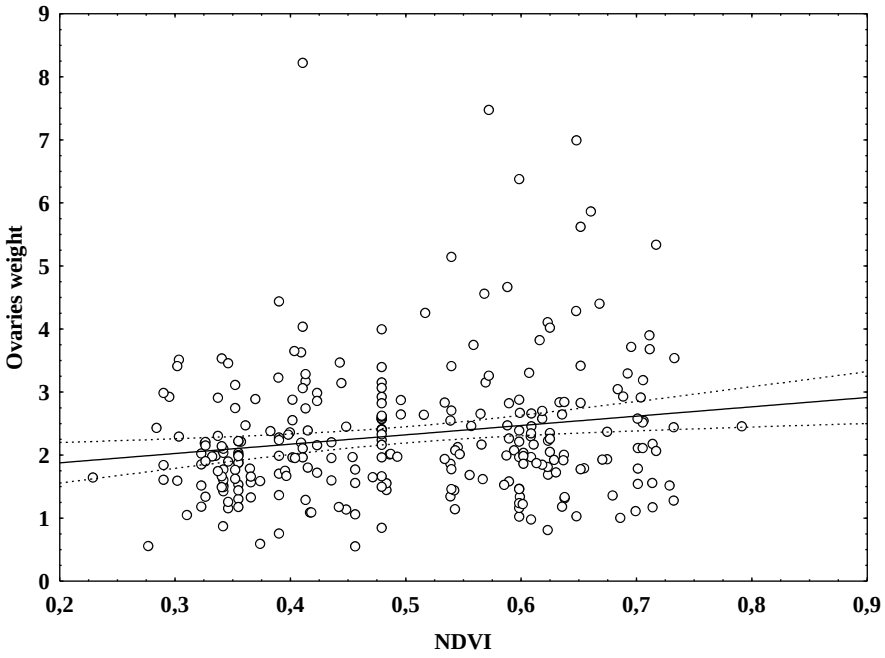

Figure 5.6. Scatterplot of 269 Egyptian mongoose ovarian weight (expressed as mg/100g of total body weight) observed for NDVI. Dashed lines denote 95% confidence intervals. Ovarian weight = 1.581+1.481x

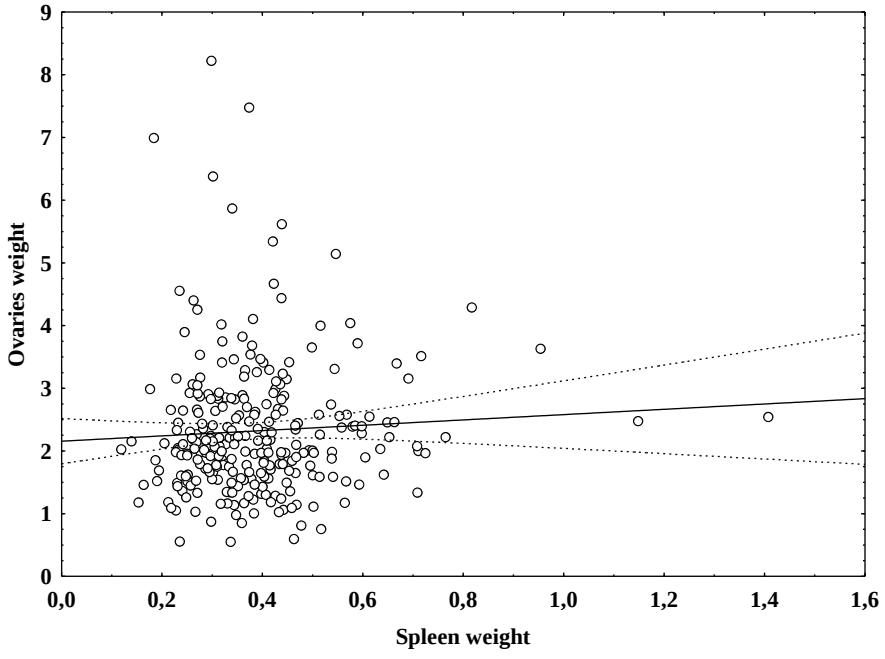

Figure 5.7. Scatterplot of 269 Egyptian mongoose ovarian weight (expressed as mg/100g of total body weight) observed for spleen weight (expressed as g/100g body weight). Dashed lines denote 95% confidence intervals. Ovarian weight = 2.1555+0.4241x

The sample contained 20 pregnant females whose gestation periods were between March and July, and a single pregnancy was registered in December (Figure 5.8). Most females were found pregnant during April (40%), followed by March (25%) May (15%), June (10%), July (5%) and December (5%) (Figure 5.8). Regarding the season, 25% of pregnant females were found in late winter, 65% in spring, 5% in early summer and 5% in late autumn (Figure 5.8). Southern females were found pregnant mainly in March and April, and those from the north in April, extending over May and June (Figure 5.9). Twenty litters were sampled from the pregnant females, most of which had three embryos or fetuses (50%), followed by litters with two (30%), four (15%) and one (5%) (Figure 5.10). The average litter size was 2.75 ± 0.79 (Figure 5.10). The northern litters had 2.70 ± 0.67 (n = 10) embryos or fetuses, whereas those from the south had 2.8 ± 0.92 (n = 10) (Figure 5.11).

A single record of a birth of four cubs in a box-trap of hunting activities was obtained, whose weights ranged between 67 g and 82 g, with an average of 75.75g. Cubs measured between 21.10 and 21.60 cm, with an average of 21.35 cm of total length (snout-tail length).

The sample contained 10 lactating females, all for the period between March and August, except April, during which there were no cases (Figure 5.12). Most females were found lactating during July (40%), followed by May and August (20% each), and finally, March and June (10% each) (Figure 5.12). Regarding the season, 10% of lactating females were found in late winter, 30% in the last months of spring and 60% in the first months of summer (Figure 5.12).

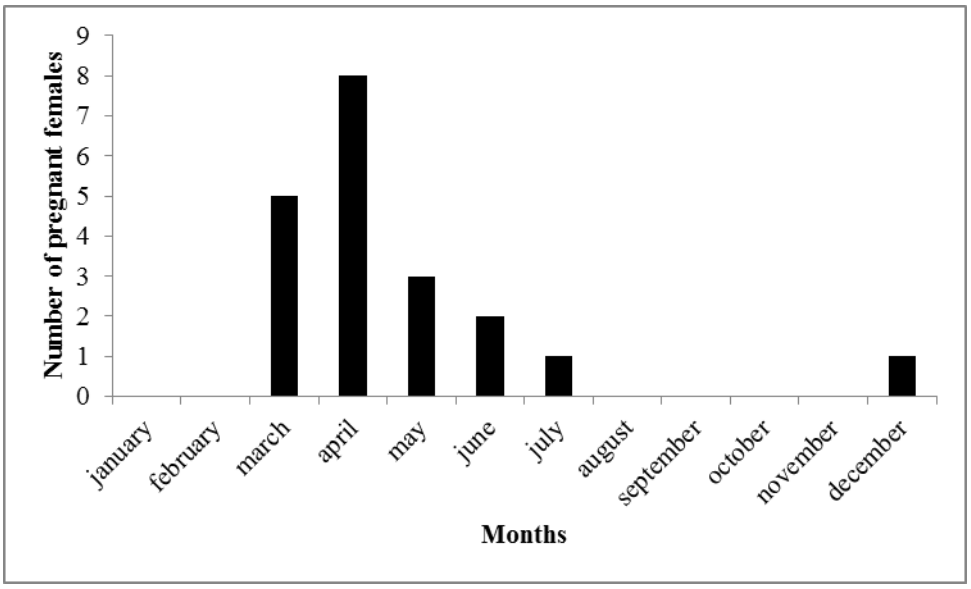

Figure 5.8. Number of pregnant females of the Egyptian mongoose by month. (n=20)

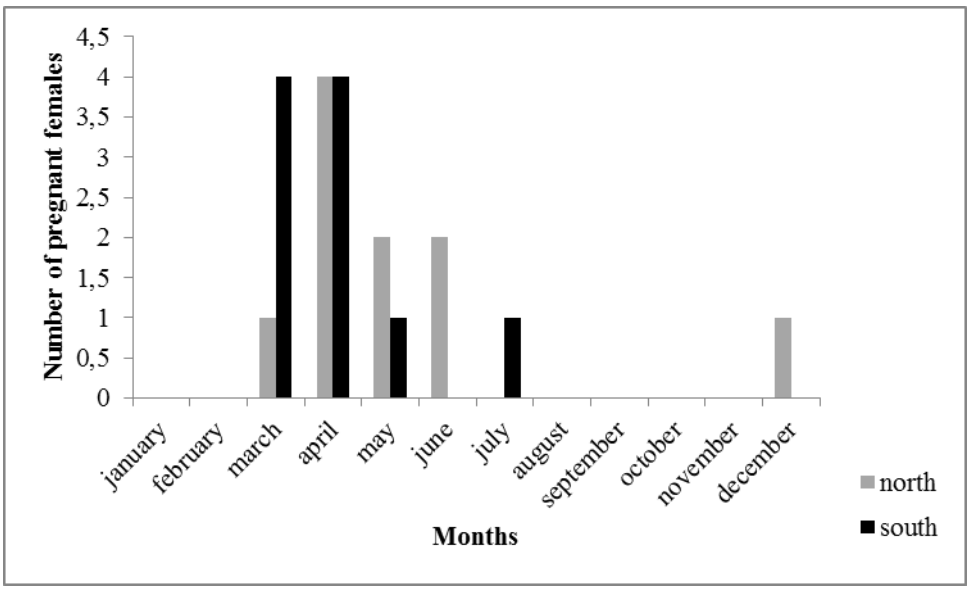

Figure 5.9. Number of pregnant females of the Egyptian mongoose among regions by month. (n=20)

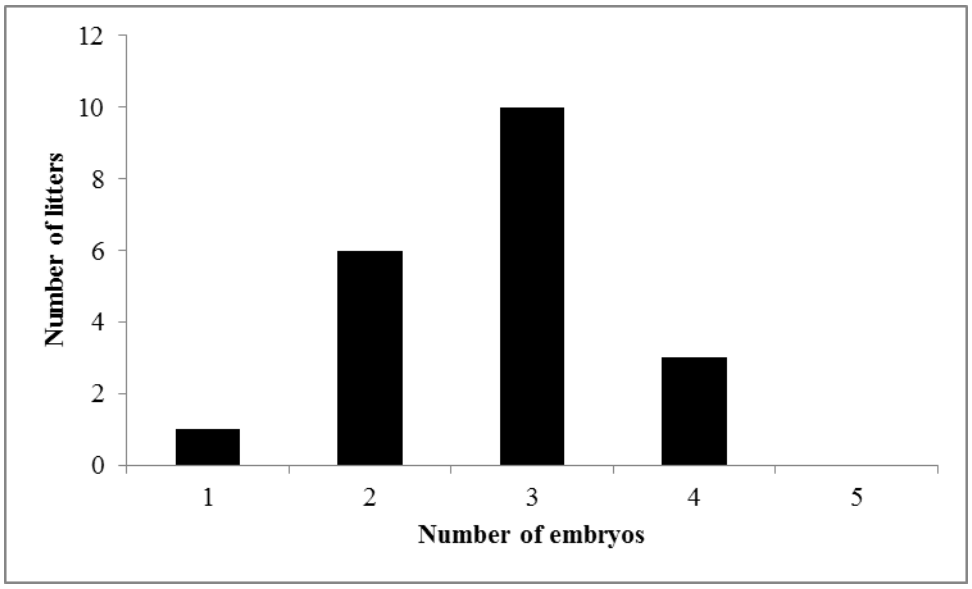

Figure 5.10. Number of litters with n embryos of the Egyptian mongoose. (n=20)

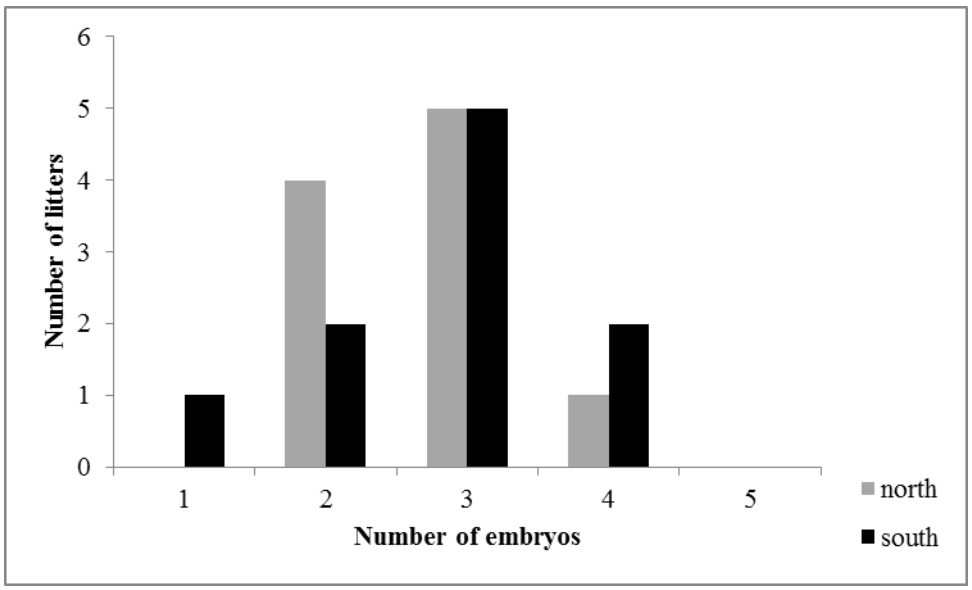

Figure 5.11. Number of litters with n embryos of the Egyptian mongoose among regions. (n=20)

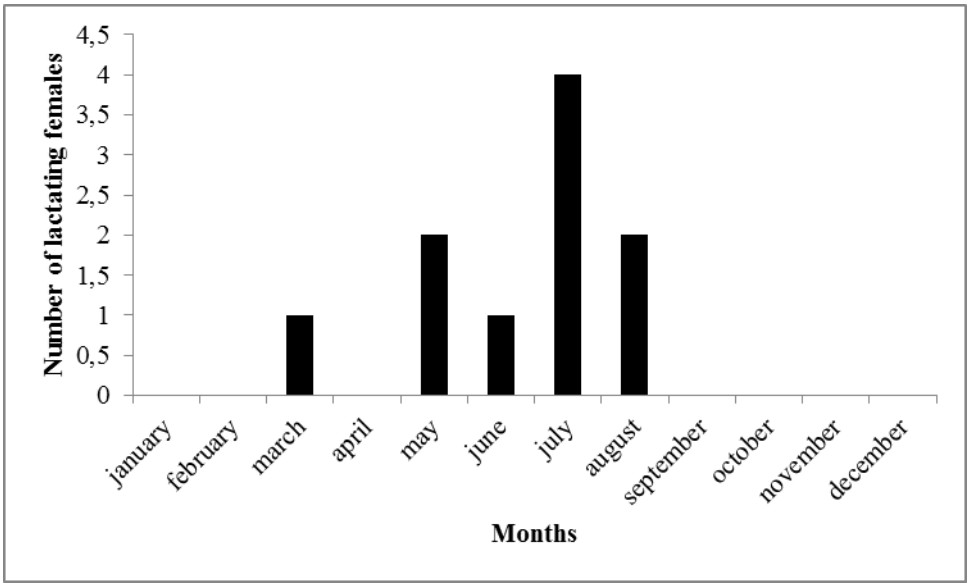

Figure 5.12. Number of lactating females of the Egyptian mongoose by month. (n=10)

Weight, length and width of adult testes showed a variation over the months of the year, with higher values observed in February, August and November (Figures 5.13 and 5.14).

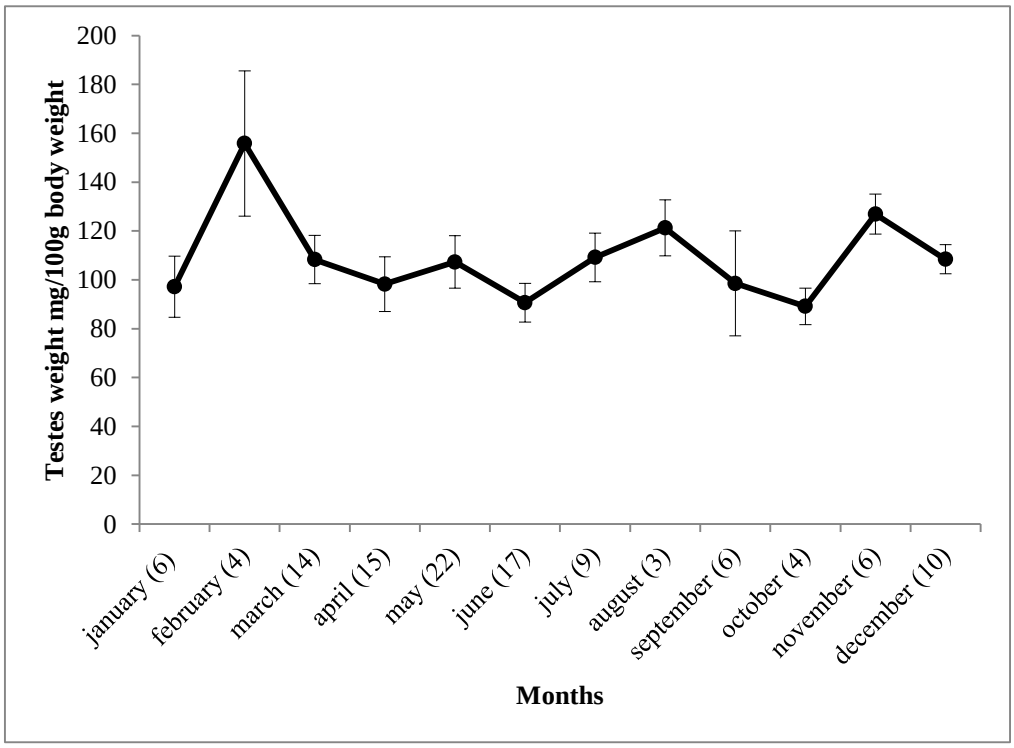

Figure 5.13. Testes weight (expressed as mg/100g of total body weight) of 116 adult males of the Egyptian mongoose over month. (n) is the number of males in each month. Vertical lines represent the standard error of the mean.

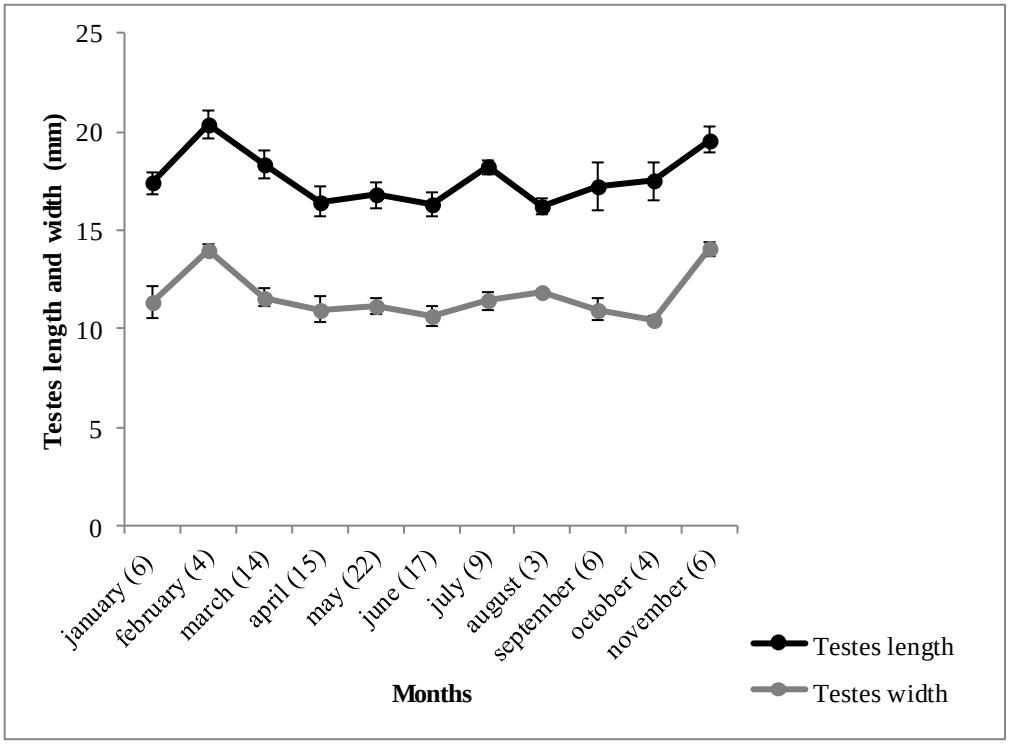

Figure 5.14. Length and width of testes of 116 adult males of the Egyptian mongoose over month. (n) is the number of males in each month. Vertical lines represent the standard error of the mean.

The GLMM analysis for testicular weight variation showed effects in age (p<0.05) and in body condition (p<0.05) (Table A4.2). After ranking all possible models using AICc (A4.3, supplementary data), two models with ΔAICc lower than two were obtained (Table 5.4). Spleen weight, season, age, NDVI and region were the variables, and season x age, season x region, and age x region were the interactions, that presented higher relative importance (Table 5.5). Males with heavier testes were found at the north of the Tagus River (Table 5.5). Adult males showed heavier testes compared to other age classes, in proportion with the increasing of age class (Table 5.5). Testes were heavier during summer (Table 5.5). Males with poorer body condition and lighter spleens had heavier testes (Table 5.5). Males with heavier testes were found in areas with lower NDVI (Table 5.5). Regarding the interaction region and age, it was found that the southern adults presented lighter testes (Table 5.5). The interaction between region and season showed that in autumn, animals from south had heavier testes (Table 5.5). The interaction between age

and season showed some seasonal variation between testes weights and the various age classes (Table 5.5). To provide some details of the factors, we refer to the averages shown Fig. 5.15 and 5.16.

Table 5.4. Models considered as explanatory on testes weight (expressed as mg/100g body weight) of the Egyptian mongoose in Portugal. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	df	AICc	ΔAICc	AICc weight
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + SEASON x AGE + SEASON x REGION + AGE x REGION	28	2181.14	0.00	0.63
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + SEASON x AGE + SEASON x REGION + AGE x REGION	27	2182.18	1.04	0.37

Table 5.5. Model-averaged coefficients for the effects of explanatory variables on testes weight of the Egyptian mongoose in Portugal.

Variables		Estimate	Std. Error	z value	Relative importance
Intercept		141.195	20.997	6.725	
SPLEEN WEIGHT		-12.502	10.446	1.197	1.00
BODY CONDITION		-0.246	2.420	0.102	0.63
	Autumn	-7.517	16.055	0.468	
SEASON	Spring	-9.517	14.564	0.653	1.00
	Summer	1.172	17.188	0.068	

	Juvenile 1	-118.790	35.524	3.344	
AGE	Juvenile 2	-69.777	23.632	2.953	1.00
	Sub-adult	-44.247	14.874	2.975	
NDVI		-3.340	24.373	0.137	1.00
REGION	South	-21.803	14.070	1.550	1.00
	South x Juvenile1	25.616	15.519	1.651	
REGION x AGE	South x Juvenile2	24.499	14.041	1.745	1.00
	South x Sub-adult	4.371	14.312	0.305	
	South x Autumn	0.663	15.813	0.042	
REGION x SEASON	South x Spring	-17.247	15.544	1.110	1.00
	South x Summer	-20.110	18.324	1.097	
	Juvenile1 x Autumn	6.760	36.720	0.184	
	Juvenile1 x Spring	32.860	34.692	0.947	
	Juvenile1 x Summer	16.520	34.125	0.484	
	Juvenile2 x Autumn	-22.028	22.276	0.989	
AGE x SEASON	Juvenile2 x Spring	-11.837	22.249	0.532	1.00
	Juvenile 2 x Summer	-20.537	21.793	0.942	
	Sub-adult x Autumn	-28.383	14.948	1.899	
	Sub-adult x Spring	-7.114	20.635	0.345	
	Sub-adult x Summer	-25.477	34.173	0.746	

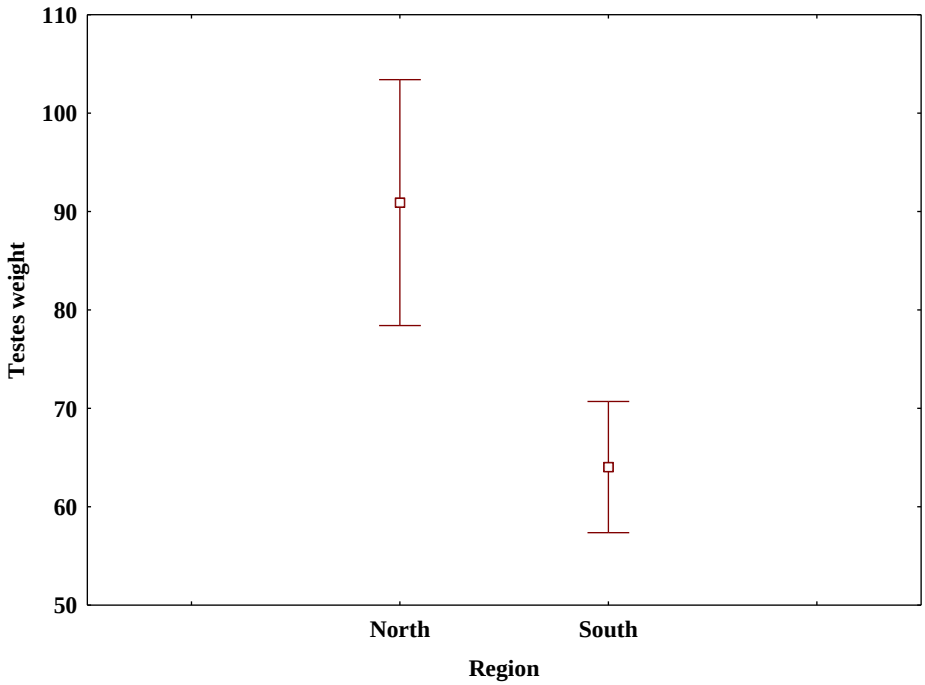

Figure 5.15. Means of 238 Egyptian mongoose testes weight (expressed as mg/100g of total body weight) across regions. Vertical bars denote 95% confidence intervals.

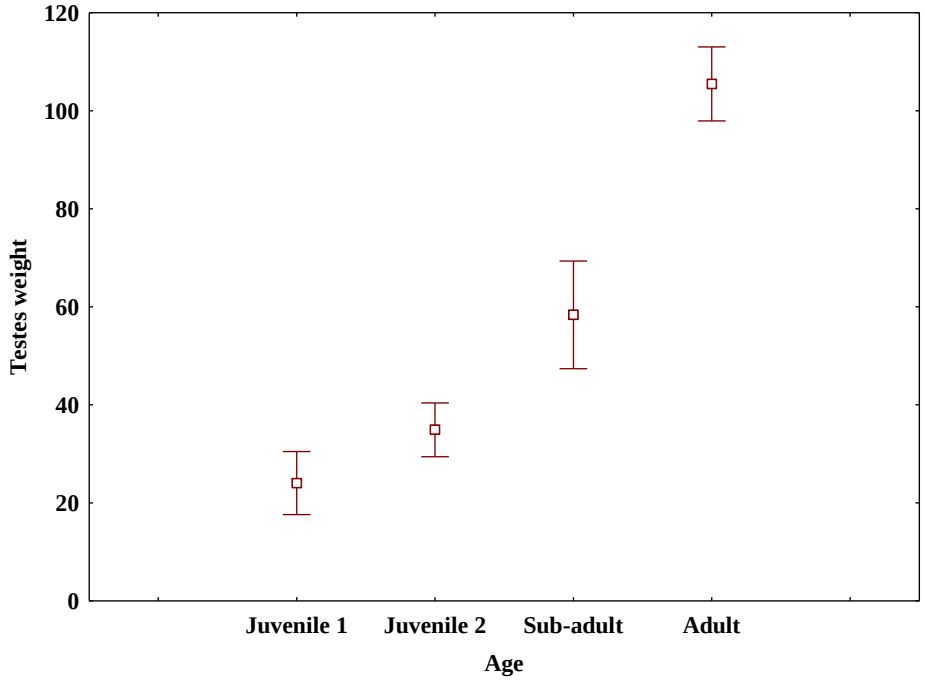

Figure 5.16. Means of 238 Egyptian mongoose testes weight (expressed as mg/100g of total body weight) across age class. Vertical bars denote 95% confidence intervals.

5.6. DISCUSSION

The variation in ovarian weight throughout the year reflected two different periods in the reproductive cycle of Egyptian mongoose females, one of activity and one of inactivity. This pattern was also identified in a closely related species, *H. auropunctatus* (Hoffmann *et al*., 1984). The active period begins in December, with an increase in ovarian mass, corresponding to the onset of ovaric activity (Carnaby *et al*., 2012), following a period of inactivity with low ovarian weights. However right after this rise, there was a decline in the ovarian mass during January, which may result from unstarted luteal activity. We assume that this species may have a desynchronization at the beginning of the reproductive activity of females, as observed in a sympatric species, the red fox (*Vulpes vulpes*), which presents a time lag in ovulation between regions (Cavallini & Santini, 1995). Thus, the reproductive activity period of the female Egyptian mongoose in Portugal is mainly between February and June, which corresponds to the period between the peak of winter and late spring, as reported for this species in Spain (Palomares & Delibes, 1992). In Israel, this active reproductive period is much shorter, occurring between February and March (Ben-Yaacov & Yom-Tov, 1983). In *H. javanicus*, it was observed that the breeding season was longer among lower population densities, with females extending the oestral cycle and permitting males to have the opportunity to copulate (Abe *et al*., 2006). Perhaps for the same reason, the Iberian mongoose populations have longer reproductive periods compared to the population of Israel. Or, perhaps habitats in Israel are worse for the Egyptian mongoose than those from the Iberian Peninsula, and this difference does not allow them to have so extensive reproductive periods in the Middle East. The counterpart *H. auropunctatus* exhibits a longer active period (February to July) (Hoffmann *et al*., 1984). Regarding the decrease in ovarian weight in July, the Egyptian mongoose displays an inactive period in terms of female reproductive organs, which extends until November. This seems to confirm that females do not usually breed throughout the year, although second mating has been registered after the loss of a first litter (Ben-Yaacov & Yom-Tov, 1983).

The active reproductive period occurs during the period of increased photoperiod and primary productivity (NDVI). Females with higher ovarian weights were found in areas with higher NDVI, corresponding to areas with greater abundance of vegetation, and

therefore greater protection, shelter and food, both for themselves and the litter, in addition to the best places for the location of burrows (Palomares & Delibes, 1990; Palomares & Delibes, 1991; Palomares & Delibes, 1993*b*; Virgós, 2001; Blaum *et al*., 2007; Santos *et al*., 2011). In this study, heavier ovaries were found specifically in a particular type of habitat, with highest NDVI values rice fields. It is expected that females with heavier ovaries and most reproductive activity will tend to occupy sites with higher productivity. At these sites, they find the most favourable places to give birth and to safely rear offspring, as well as higher density of prey. Thus, they are able to avoid prolonged absence from the offspring, as well as wasting energy reserves necessary for lactation. This same kind of preference for the more productive places is evidenced by female Eurasian otter (*Lutra Lutra*), in which males occupy the least productive areas (Kruuk, 1995; Ruiz-Olmo *et al*., 2005). The female Eurasian otter selects areas with adequate and abundant burrows, and high availability of water and prey, around which they can rear the cubs, functioning as an energy-saving strategy, reducing its movements and vulnerability, which in turn may maintain or increase the reproductive success (Kruuk, 1995; Ruiz-Olmo *et al*., 2005). Therefore, it is possible that a similar spatial segregation and habitat selection exists between sexes, that would be related to productivity, with male mongooses showing more flexibility on habitat selection and females being more rigid to locate their home ranges in the best and more productive habitats.

Reproduction and survival require energy investments that may place these two processes in competition with each other, in which an increase in reproductive investment can suppress or hinder immune function for self-protection (Zuk & Stoehr, 2002; Stoehr & Kokko, 2006; French *et al*., 2007). An increase in energy demand by the immune system caused by disease or parasitism may involve the reduction of reproductive performance, at least for some individuals, because those individuals in better condition can accomplish the different life-history processes without detrimental effects (Goossens *et al*., 1997; Zuk & Stoehr, 2002; Stoehr & Kokko, 2006). Therefore, immune-reproductive/survival trade-offs can be very different within and among populations depending on how variance in condition is distributed among individuals. To increase the survival of a species that has a long lifespan, development of a strong immune competence can be a good strategy, crucial to the reproductive success, because the maximum fitness is obtained when an individual survives through several reproductive events (Lochmiller & Deerenberg, 2000), but only

individuals in good condition can accomplish at the same time strong immunity and survival linked to health, especially during all lifespan. If individuals are in lower condition, during the reproductive period, the allocation of energy to reproduction imposes costs that can hinder the immune response (Lochmiller & Deerenberg, 2000; French *et al*., 2007) and this can compromise survival and ultimately lifetime reproduction. As the spleen weight may reflect the immune capacity, it is expected that an individual with greater spleen mass may have greater capacity for immune response (Fernández-Llario *et al*., 2004; Goüy de Bellocq *et al*., 2007). Thus, an enlarged spleen weight accompanied by a decrease in ovarian weight may reflect a negative trade-off between reproduction and immune function during the reproductive period (French *et al*., 2007). However, our results showed that the overall trend in mongooses is that those individuals with heavier ovaries also have heavier spleens suggesting that the negative predicted trade-off was not found in Egyptian mongoose females, and presumably the best fitted animals can reproduce and maintain immune competence without causing prejudice to each other.

The pregnant females were found in late winter, spring and early summer, corresponding to the period where ovarian weight records were higher and consequently to the active reproductive period. This suggests that in most cases Egyptian mongoose females produce only one litter per year during the same period as other sympatric carnivores (Ruiz-Olmo, 1997). While in the south, the pregnant females were most frequent in the months of March and April, the northern pregnancies seem to be further distributed throughout spring, maybe linked to lower habitat quality which requires extend reproductive period to guarantee successful matings when populations are of lower population size. One of the females of the northern region was found pregnant during December, and may be an early female, since luteal activity was observed in this month, or it may be the first litter from a late primiparous female (Ruiz-Olmo, 1997). Litter size can range from one to four cubs, as in Israel (Ben-Yaacov & Yom-Tov, 1983), with three as the modal value, which seems to contradict the widespread idea of multiple large litters. Litters of the southern region seem to be larger than those from the northern region, as was expected because the presumed higher habitat quality of south of Portugal, where mongooses reached higher density and show overall better condition (Bandeira *et al*., 2016; Bandeira *et al*., *submitted*). Indeed, for most species, fitness related variables are higher in optimal habitats in the center of the distribution (Fuller & Sievert, 2001; but see Sagarin & Gaines, 2000). Alternatively, if the

populations in core areas can live under large competition among individuals, negative density-dependent processes can produce a reduction in fitness elements. For example, a study that evaluated the reproductive response of *H. javanicus* of a Japanese island to population control described that the average litter size was significantly higher in populations for individuals living on the periphery of the distribution of the species, where the density was lower (Abe *et al*., 2006). It has been observed that after controlling actions in *H. javanicus*, the litter size and the reproductive period increased, due to better nutritional conditions resulting from decreased competition between individuals (Abe *et al*., 2006). This aspect is important to monitor in *H. ichneumon*, as in Portugal the populations are indeed subject to density control. Litters in Portugal have a tendency to be on average lower than those from Israel (Ben-Yaacov & Yom-Tov, 1983), but larger and with a wider range than those from south Spain (Palomares & Delibes, 1992; Palomares, 1993*b*).

The lactation period in females occurs from late winter to the peak of summer, which confirms that births can begin at least in March, earlier than females from southwestern Spain (Palomares & Delibes, 1992; Palomares, 1993*b*). In addition, there is no occurrence of lactating females in September, so it is assumed that at this time there are no longer cubs in dens and that the offspring already accompany the mother in search of food, taking into account the descriptions of larger groups observed from July in other populations (Ben-Yaacov & Yom-Tov, 1983; Palomares, 1993*c*; Palomares & Delibes, 1993*a*). Regarding the period of emergence of lactating and pregnant females, it is anticipated that the Egyptian mongoose mating season in Portugal begins at least in February, and possibly up until June, similar to the southwestern Spain population (Palomares & Delibes, 1992; Palomares, 1993*b*), a rather longer period than that observed in Israel (Ben-Yaacov & Yom-Tov, 1983). A delay in reproduction may cause cubs to reach winter at a young age (Cavallini & Santini, 1995).

Egyptian mongoose males did not show a marked reduction in testicular weight during autumn. However, fluctuations were observed in the weight of the male reproductive organs throughout the year, as opposed to the males of *H. auropunctatus* that also exhibit two breeding periods, an active and an inactive (Soares & Hoffmann, 1981; Soares & Hoffmann, 1982). The results of this study indicate that the Egyptian mongoose males are

able to reproduce all year (Palomares, 1993*b*), despite a peak in testicular weight in February, which coincides with the beginning of the main period of increased ovarian mass and therefore luteal activity in females. Thus, it is suggested that the beginning of the main breeding period of the Egyptian mongoose in Portugal is during February, in the peak of winter, with the increase of photoperiod and primary productivity. We speculate that males do not have a breeding season as well defined as that of females, which are responsible for the timing of reproduction in this species, as occurs with the sympatric species Eurasian badger (*Meles meles*) (Yamaguchi *et al*., 2006).

The adult male mongooses presented heavier testes in relation to sub-adults and juveniles, such as in red fox (*Vulpes vulpes*) (Cavallini & Santini, 1996); as opposed to the weights of the female reproductive organs, which did not differ among age classes.

Males with poorer body condition and lighter spleens had higher testicular weight, an indicator of spermatogenic activity (Cavallini & Santini, 1996; Ruiz-Olmo, 1997), suggesting a trade-off between reproduction and immune competence in this gender (Zuk & Stoehr, 2002; Stoehr & Kokko, 2006), which contrasted with the observed in females. Therefore, trade-offs appeared to change between genders reflecting the different life history strategies in relation to reproduction. Males in weak physical conditions may allocate their resources to reproduction, in the detriment of immune function (Zuk & Stoehr, 2002). Although animals of the northern region have smaller body size (Bandeira *et al*., 2016), they presented highest testicular weight, indicating higher spermatogenic activity, because there is less competition between individuals in the region and lower density (Abe *et al*., 2006). Males with heavier testes were found in areas with lower primary productivity, perhaps because they travel longer distances to defend territories and mate with several females (Palomares, 1993*a*; Palomares & Delibes, 1993*a*; Palomares & Delibes, 1993*b*; Palomares, 1994), thus moving away from the more sheltered locations, that are selected by females, due to imposition of their own sexual dimorphism in habitat use, as seen in Eurasian otter (Kruuk, 1995; Ruiz-Olmo *et al*., 2005).

Our study suggests that females of Egyptian mongoose have a highly seasonal reproduction, although males are able to reproduce all year. Reproduction in females appears to be mainly influenced by season and is regulated by increasing photoperiod. Apparently, females balance the capacity for reproduction as well as for the immune

function. In opposite, males, beyond seasonality, are also greatly influenced by the conditions of the region where they live, by their territorial success and mating strategy that can encompass several females, as well as with immune function and physical condition that evidenced a trade-off with reproduction costs.

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CHAPTER 6

HIGHLIGHTS ON BIO-ECOLOGY OF THE EGYPTIAN MONGOOSE IN PORTUGAL

6. HIGHLIGHTS ON BIO-ECOLOGY OF THE EGYPTIAN MONGOOSE IN PORTUGAL

The methods used, the analyses performed and the results obtained herein were reported and discussed in detail throughout the various chapters of this thesis. Here, we summarize the main findings and their bases, and various suggestions set out along this thesis in the form of highlights, which convey the key findings.

CHAPTER 2

- For the first time, sexual dimorphism in body size of adult Egyptian mongoose in Portuguese territory was reported.

- Adult males are significantly larger than females in main external biometric parameters and weight.

- Animals established in the south of the Tagus River have larger body size than those from the north.

- The main external body measurements and weights of Egyptian mongoose were described for different age classes based on an objective dentition-based age classification. For the first time, the main external body measurements and weights of juvenile type I and type II, and sub-adult mongoose', were described.

- Differences between genders, ages and regions suggest that body size in this species is regulated by sexual selection, food availability, human pressure and by different habitat usage patterns.

- Adults of Egyptian mongoose in Portugal exhibit lower body mass than animals from Doñana (Spain) and Israel.

- Adults of Egyptian mongoose in Portugal exhibit longer body length than animals from Doñana (Spain) and Israel.

CHAPTER 3

- The variation in spleen weight was described for the first time in Egyptian mongoose.
- Males' spleens are heavier than females' spleen.
- Spleen weight peaks during winter and spring in this species.
- Variations of the spleen weight may be due to reproductive behavior, parasitism or disease.
- Body condition was described in a large sample of Egyptian mongoose, using a body condition score.
- Body condition is higher in males.
- Body condition is higher in adults.
- Variations in body condition may be due to the diet, reproductive ecology, or immune competence.
- The relation between spleen weight and body condition was assessed. Spleen weight increases with the increase of body condition.
- Spleen weight and body condition are mostly influenced by life-history traits and gender-specific differences rather than the influence of habitat, primary productivity, rainfall or temperatures.

CHAPTER 4

- The Egyptian mongoose feeds mainly on mammals, followed by reptiles, amphibians and invertebrates.

- Males consume more mammals and amphibians, while females, consume more reptiles and invertebrates.

- Diet varies between north and south of the Tagus River regions.

- Diet varies throughout seasons, depending on prey availability and on (seasonal) energetic needs.

- The consumption of more energetic species, such as mammals and reptiles, occurs during spring.

- Mammals, amphibians and invertebrates are obtained in areas with higher primary productivity, while reptiles in drier areas.

- Specimens with smaller body size consume more invertebrates.

- The specimens with heavier spleens consume more mammals and reptiles, corresponding to the most consumed items by males and females, respectively, during the spring, when the organ's mass peaks.

- Animals with better physical condition consume more mammals and amphibians, corresponding to the most consumed items by males.

- Combining all data and results gathered, lead us to hypothesize that the consumption of more energetic prey, such as mammals, results in better body condition and heavier spleens, possibly driving to a greater investment in immunity.

- Egyptian mongoose do not rely specifically on game species. Their consumption is optional and only really important in areas where these species reach high densities.

- This study reported three new species of reptiles in the Egyptian mongoose' diet, as well as four new locations of three species of reptiles in Portugal.

- The expansion of the Egyptian mongoose to the north can potentially endanger some populations of endemic species and possibly cause some impact on conservation in this region due to resource competition.

- The digging and exploratory behavior of the Egyptian mongoose seems to allow it to take advantage on the activity peaks or less mobility of each category of consumed animals.

CHAPTER 5

- Females have two periods in their yearly reproductive cycle, an active period, between December and June, with a peak in reproductive activity February, and an inactive period, between July and November.

- Males seem to be reproductively active throughout the year, with a peak in spermatogenic activity in February.

- The weights of the reproductive organs, corrected for body mass, of males and females showed a simultaneous peak of activity in February.

- Females with higher ovarian weights have heavier spleens, and are found in spring, in places with higher primary productivity.

- Males with larger testicular weights are adults, with lighter spleens, poorer body condition, and found in the northern region, in places with low primary productivity, during the winter season.

- Pregnant females are found between March and July, and there may be exceptions.

- Lactating females are found between March and August.

- Average litter size is 3 cubs ($\bar{x}$=2.75), 3 is the mode, and a range of 1 to 4 cubs.

- Female reproduction seems to be mainly influenced by season, due to regulation by the increased photoperiod.

- Reproduction of males is dependent on factors beyond seasonality, among them, the region, territories with access to several females, immune competence and physical condition.

- Females with heavier ovaries were found in places with higher primary productivity, while males with heavier testes lived in poorer habitats, with sexual dimorphism on habitat selection.

- In females, heavier ovaries and heavier spleens are positively correlated, suggesting that the most fitted animals can balance reproduction and maintain immune competence without abolishing each other. In contrast, a trade-off between body condition and reproduction performance was verified for males, whose worse body condition and lighter spleen mass correlated with higher testicular weight.

CHAPTER 7

FINAL CONSIDERATIONS
AND
FUTURE PERSPECTIVES

7. FINAL CONSIDERATIONS AND FUTURE PERSPECTIVES

This final chapter gathers the information that is not in the articles and other thesis chapters. It includes the lessons learned from situations and obstacles that arose throughout the work, which allowed the adaptation and improvement of the methods, in hope that this information may prove useful or interesting to those reading this thesis or working in related fields.

The use of wild animal carcasses obtained from hunting activities, or from accidental road kills, is a valuable resource for the study of the ecology of the Egyptian mongoose. This inexpensive and non-invasive procedure allowed contributions to the knowledge on its fundamental, trophic and reproductive ecology. The study of the dead mongoose allowed an increase in the knowledge of the remaining populations.

The number of samples collected in northern and southern regions showed a variation. Animal censuses indicate that the Egyptian mongoose is found in highest densities in the southern region (ICNF *unpublished data*), hence the collected samples of the north account for only 1/4 of the total sampling size. The disparity in the number of animals collected between the two regions may also be due to different capture efforts, because this species is best known by the hunting community in the southern region, where predator control events are more widespread. Additionally, higher densities in the south are likely to increase the probability of road kills. The number of samples taken *per se*, also reflects the abundance of Egyptian mongoose present in the two study regions.

About a hundred collected animals collected were rejected from this study. These animals were excluded due to advanced state of decomposition, due to the long period elapsed since the death to freezing, which resulted in a high level of autolysis, and, in these cases, only external biometric parameters were recorded. Other specimens were excluded because they were incomplete and very damaged, mainly the carcasses from road accidents. Some specimens, whose skull was removed during sampling, could not be analyzed in terms of tooth development in order to assign an age class, and as such, they were also excluded.

The fundamental ecology of the Egyptian mongoose based on measurement of main biometrics and on the record of the weight of each carcasses, allowed to describe the variation and the average in each age class of the two genders, covering a wide distribution

involving the main habitats of the two biogeographic regions of the Iberian Peninsula, where this species is present. This is the first research report that reveals the average weights and the main external body measurements of juvenile I, juvenile II and sub-adult stages of Egyptian mongooses in the Iberian Peninsula, useful in publications directed toward civil society, such as mammal field guides, nature documentaries, and information panels about the species. This work describes the biometric parameters of juveniles and sub-adults, using an age classification based on a rigorous yet simple dental development analysis, after an extraction of surrounding tissues, which are degraded through immersion on an enzymatic solution. This process involved the analysis of deciduous and definitive teeth (Figure A5.1, supplementary data), such as the four canine teeth, cross-referenced with information already reported by Palomares *et al*. (1992), and Ben-Yaacov & Yom-Tov (1983). This combination of data allowed the division of juveniles into two classes: type I and type II. This division allowed finer data, taking into account that the juvenile period as a whole lasts about seven months. However, while apparently short, this period involves large variations in dimensions, energy states, immune competence, behavior and diet. The results obtained in this study underline the importance of this age division. Intersecting all the biometric and weight data for the early stages indicates more sexual variation during the sub-adult stage, in comparison with the previous age classes. In the sub-adult period, the trend is that females are slightly larger than males. However, it should be noted that these results could reflect different maturational stages and sexual hormonal conditions of specimens inside this age class, thereby affecting growth levels of different genders (Badyaev, 2002). In addition, based on the observations of under-developed canine teeth at this age, it is possible that it could influence capture and the consumption of prey, which, in turn, would create constraints on growth. When considering juvenile type I Egyptian mongooses, no differences in weight and biometrics were registered between genders, suggesting similar energy intakes. As juveniles in this initial stage do not capture prey, the entire litter is completely dependent on food provided by mother, supposedly leading to the same food intake (Ben-Yaacov & Yom-Tov, 1983). In the next phase, a trend begins to appear in juveniles type II, when they capture their own prey (Ben-Yaacov & Yom-Tov, 1983). Assuming males are able to capture larger prey, there should be a tendency for differentiation in body biometrics and mass, due to access to different food items, and different levels of energy for growth (Ralls & Harvey, 1985) that, at some point,

is consolidated with adult males capturing and consuming larger prey (Rosalino *et al*., 2009). The same happens in species that are dimorphic since birth, such as *Mustela vison*, whose food availability is similar at that stage (Thom *et al*., 2004). Thus, in the case of mongooses, other factors besides food availability need to be considered for the lack of sexual dimorphism at birth and the first stages of development. Indeed, in most vertebrates the normal rule is that genders are similar at birth and in the first periods of growth, and they only diverge when entering the adult stage (Badyaev, 2002). The main developmental mechanisms that produce sexual size dimorphism in most species is related to gender specific differences in growth rate and growth duration, in whom expression of morphological variation can depend on the concentration, synthesis, secretion and sensitivity of growth hormone during development (Badyaev, 2002). The assignment of age in years to adults was delayed because counting of *cementum annuli* rings deposited annually (eg. Stoneberg & Jonkel, 1966; Root & Payne, 1984; King, 1991) still needs to be validated for this species. The determination of the age class of each individual through the confirmation of tooth development, corrected mistakes made during the initial observations of carcasses, on the dissection table, when a first approximation of the age (age class) was assigned. At first sight, the weights and the body size of the animals, as in the case of some juveniles, had parameters similar to adults, and vice versa, inducing erroneous age classifications when based only on external morphology. Thus, the work developed in this thesis illustrates the advantages that the age class in wild carnivores' carcasses should be confirmed after cranial cleaning by enzymatic action to analyze tooth development.

Agreements with other research groups involved sharing parts of tissues or organs of collected Egyptian mongoose. Thus, some of the shared organs, such as spleen fragments, resulted the loss of data, for example where the use of the complete organ to determine the ratio of the two parenchymas (see Corbin *et al*., 2008) would be helpful to establish a relation between spleen weight and immune competence. However, combination of the obtained data with the use of shared samples, will be an added value to the continuity of the Egyptian mongoose ecology study in Portugal. The results obtained from this thesis, combined with the data provided by the laboratories associated with this project, which are analyzing heavy metals accumulation, pathogen circulation, abundance, burden and genetic relatedness, other aspects related to health, and population genetic analysis, is perhaps a major breakthrough where the highest maximization of samples enabling the highest

combination of data may advance current knowledge well beyond, from the same sampling effort. Some of these partnerships have already yielded results, namely in sanitary status (Duarte *et al*., 2013; Cumbassá *et al*., 2015, Reis *et al.*, submitted, Cunha *et al*, submitted), in the exposure to heavy metals (Rodrigues *et al*., 2014), or in landscape genetics (Barros *et al*., 2016*a*, 2016*b*).

The diet is one of the best studied ecological aspects in the Egyptian mongoose, due to ease in obtaining samples of faeces or stomach contents. However, some concerns remain within the hunting community, which believes this mongoose to prey and consume European rabbit (*Oryctolagus cuniculus*) and red-legged partridge (*Alectoris rufa*) and its eggs, as the basis of its diet. One of the objectives of this study was to obtain robust data to better understand the trophic ecology of the Egyptian mongoose throughout the Portuguese territory, both in its original distribution, and in the expansion areas. The results indicated that, although the Egyptian mongoose includes these game species in its diet, they account for less than half of biomass consumption, within a diverse spectrum of food items. These results were based on data obtained from the stomach contents, with several advantages over the study of intestinal contents or faeces. The analysis of stomach contents is preferred since they are less degraded, allowing the identification of fruits, plants, invertebrates, amphibians, eggs, and the assessment of the number and size of ingested prey items, the differentiation of carrion from live prey (that in the intestinal contents would be very difficult to recognize), as well as a more reliable value of consumed biomass than in faeces (eg. Britton *et al*., 2006; Balestrieri *et al*., 2011). However, a limitation recorded during this work was that a large part of the stomachs were empty, because the retention time of stomach contents is too short to obtain high numbers of samples with contents. Furthermore, due to limited number of juveniles and sub-adults found with stomach contents, it would be important to increase the sampling in the future, in order to be able to make more robust inferences about juvenile age groups, where we would expect, for example, juvenile type I to present a higher consumption in invertebrates. The analysis of stomach contents revealed the inclusion of new species of reptiles in the Egyptian mongoose's diet and new locations of reptile species in Portugal. The assignment of the location of each reptile was estimated based on the location of the respective mongoose with the UTM grids of maps of the Atlas of Amphibians and Reptiles of Portugal (Loureiro *et al*., 2008). Given that the location for the collection and date of

death of each mongoose is known, and that these animals live in areas with about 3 km^2 (Palomares & Delibes, 1991), covering 4 to 5 km per day (females and males, respectively) (Palomares & Delibes, 1998), four individuals in this study were found to contain three species of reptiles collected in geographical areas whose location was not yet marked on the maps of the Atlas of Amphibians and Reptiles of Portugal in their stomachs (Figure A5.2, supplementary data). The new locations refer to Slow Worm (*Anguis fragilis*), Western Three-toed Skink (*Chalcides striatus*) and Southern Smooth Snake (*Coronella girondica*). In addition to these new locations, Slow Worm (*Anguis fragilis*), Horseshoe Whip Snake (*Hemorrhois hippocrepis*) and Southern Smooth Snake (*Coronella girondica*) were reported in the Egyptian mongoose diet for the first time.

In light of current knowledge, this was the first study in Portugal which was dedicated to the reproductive ecology of the Egyptian mongoose. This study contributes to the knowledge of reproductive ecology of the Egyptian mongoose by identifying the periods of the year in which they are fertile, pregnant and lactating, as well as the size and frequency of litters. These results do not confirm the existence of multiple and numerous litters. In the future, we expect to perform histological evaluations of all reproductive organs to study the level of development in each gender, age, season and region, and thus refine the results on its reproductive ecology.

The information obtained from this research can provide a baseline for future scientific projects related to genetics, health, or climate change; it might also contribute to a review of hunting status of the Egyptian mongoose in Portugal; and promote knowledge basis for the management actions undertaken by the hunting community in our country. The largest collection of Egyptian mongoose samples of the world gathered over the past few years in the Department of Biology of the University of Aveiro holds potential to further contribute to the knowledge of the ecology of this species, which ultimately may be extrapolated to other top predators, namely of the *Herpestidae* and beyond.

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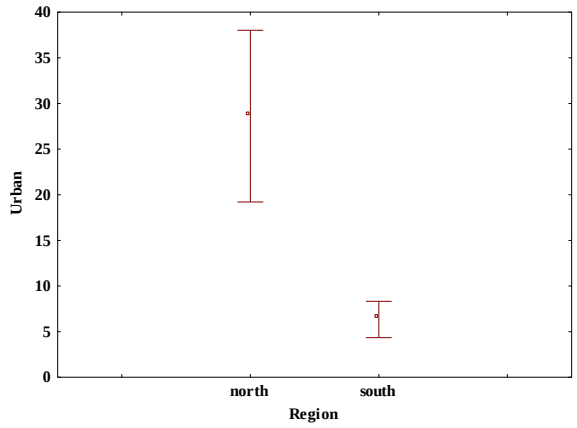
SUPPLEMENTARY DATA

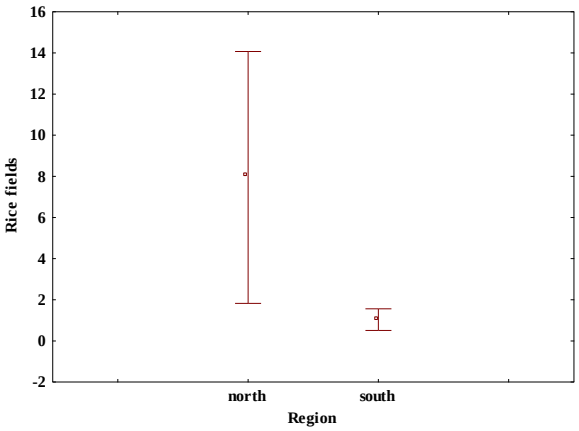
APPENDIX A1

Table A1.1. Loadings for each variable of body measure in PCA.

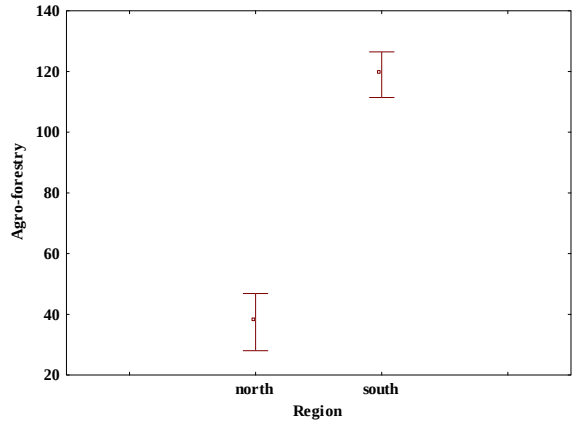
Variable	Values of factor loadings
Body Weight	-0,925218
Snout-Tail Length	-0,911656
Right Hind Leg Length	-0,881857
Right Hind Foot Length	-0,858199
Shoulder Height	-0,729124
Neck Perimeter	-0,904782
Head Width	-0,770564
Eigenvalue	5,145347
Proportion of variance	0,735050



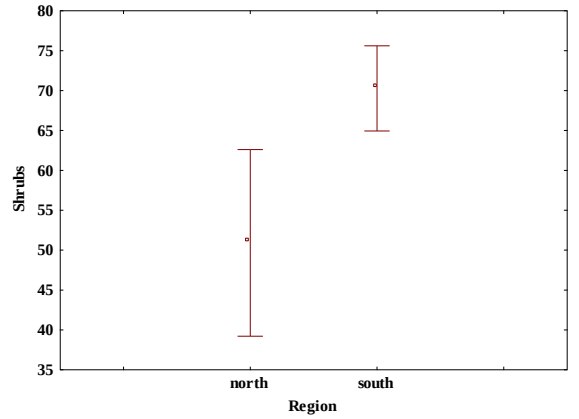
A - Urban



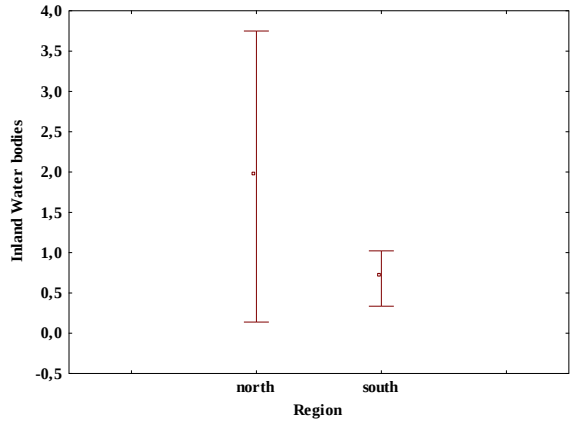
B – Rice fields



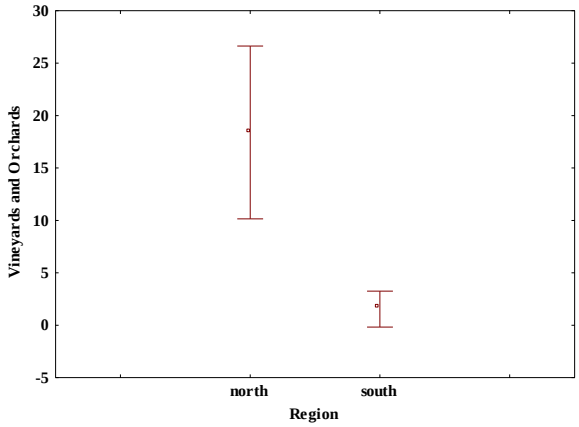
C – Agro-forestry



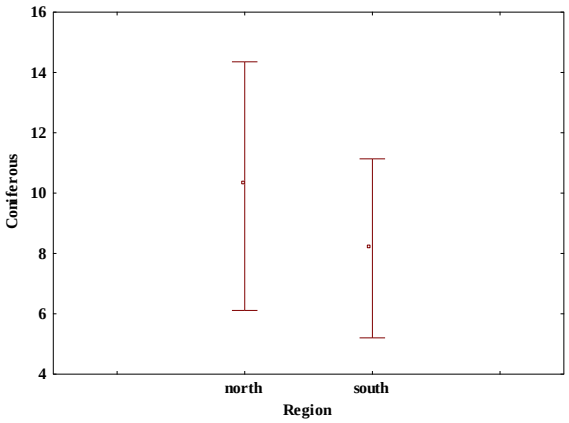
D - Shrubs



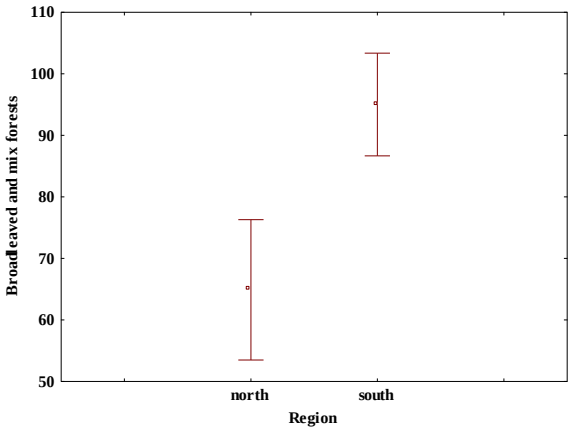
E – Inland water bodies



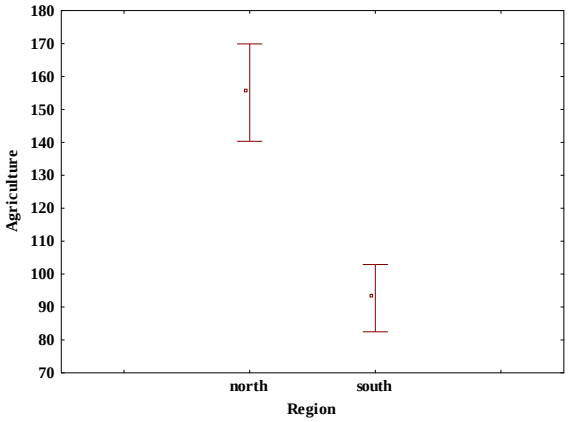
F – Vineyards and orchards



G - Coniferous

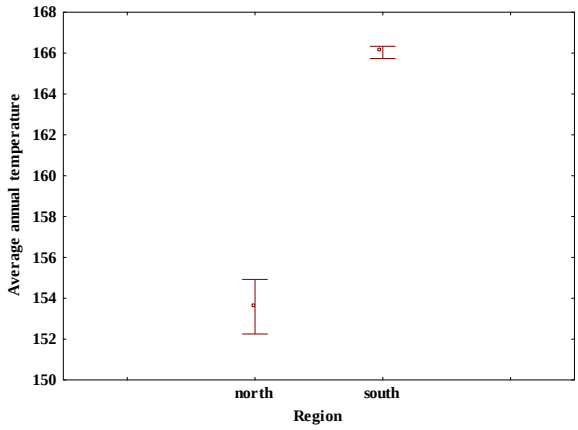


H – Broadleaved and mix forests

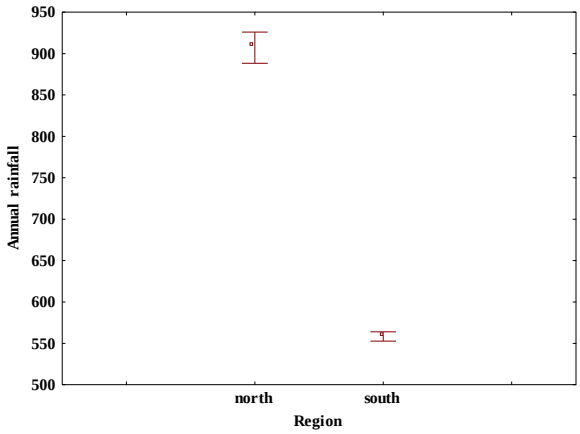


I - Agriculture

Figure A1.1. Means of number of hectares of urban, rice fields, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, and agriculture areas observed for each region of Egyptian mongoose sample location. Vertical bars denote 95% confidence intervals.

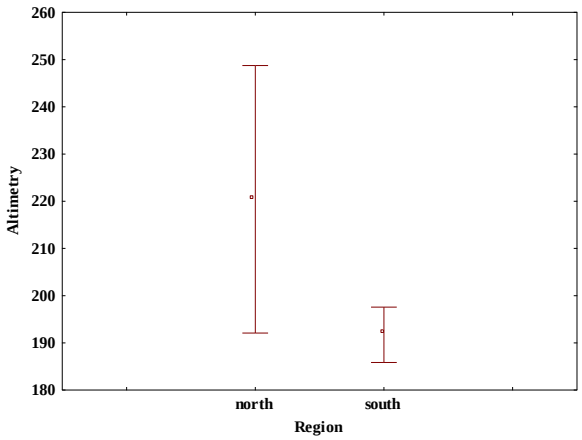


J - Average annual temperature

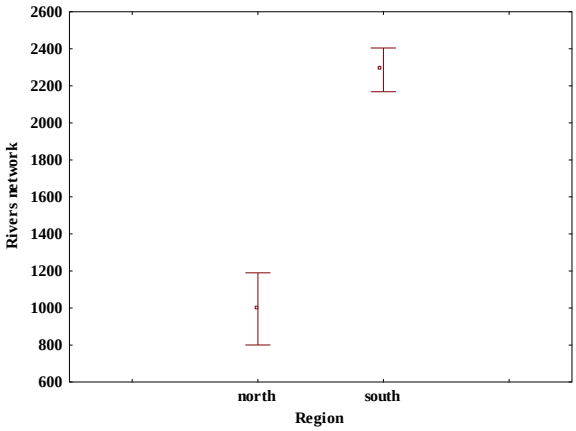


K - Annual rainfall

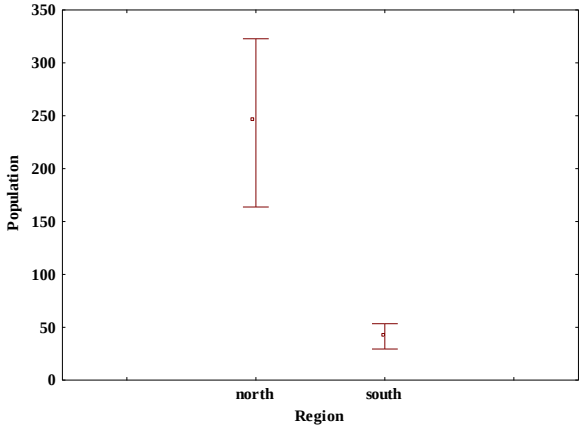
Figure A1.2. Means of average annual temperature and annual rainfall value observed for each region of Egyptian mongoose sample location. Vertical bars denote 95% confidence intervals.



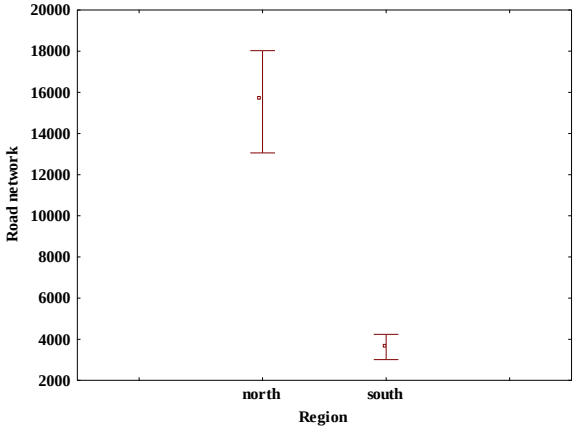
L - Altimetry



M – Rivers network



N - Population



O – Road network

Figure A1.3. Means of altimetry value, distance in meters of river network, number of inhabitants per km^2, distance in meters of road network, observed for each region of Egyptian mongoose sample location. Vertical bars denote 95% confidence intervals.

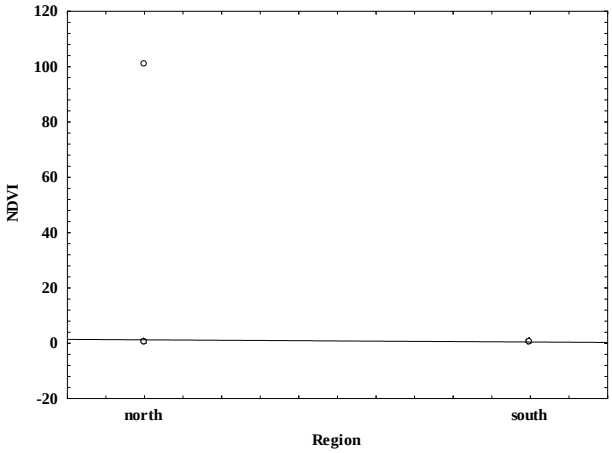


Figure A1.4. Scatterplot of NDVI (primary productivity) observed for each region of Egyptian mongoose sample location. NDVI = 81.0499-0.7901*x

APPENDIX A2

Table A2.1. Results obtained from GLMM analyses to examine gender, age, region and/or season variables as categorical predictors of spleen weight (expressed as g/100 g body weight) of Egyptian mongoose. District was used as random factor to control for spatial autocorrelation of samples. Subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI), body size (PCA1 of all measured biometrics and weight) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. [df – degrees of freedom; MS – mean square; F – statistic value; *p* – corresponding significance value. (*p* significant values at the 0.05 level are in bold).]

Effect	**df**	**MS**	**F**	***p***
Intercept	1	0.015	0.389	0.5330
District	11	0.084	2.242	**0.0118**
Region	1	0.078	2.069	0.1511
Season	3	0.105	2.800	**0.0397**
Gender	1	0.030	0.792	0.3739
Age	3	0.041	1.090	0.3532
Region×Season	3	0.005	0.126	0.9447
Region×Gender	1	0.052	1.371	0.2422
Region×Age	3	0.026	0.679	0.5652
Season×Gender	3	0.068	1.793	0.1479
Season×Age	9	0.040	1.051	0.3986

Gender×Age	3	0.154	4.088	**0.0070**
Region×Season×Gender	3	0.002	0.058	0.9815
Region×Season×Age	7	0.011	0.296	0.9552
Season×Gender×Age	9	0.068	1.806	0.0653
Region×Gender×Age	3	0.004	0.094	0.9634
Region×Season×Gender×Age	4	0.009	0.252	0.9083
Subcutaneous fat	1	0.028	0.738	0.3908
Visceral fat	1	0.018	0.475	0.4912
Egyptian mongoose abundance proxy	1	0.187	4.957	**0.0265**
European rabbit abundance proxy	1	0.281	7.468	**0.0065**
Red-legged partridge abundance proxy	1	0.232	6.158	**0.0135**
Urban	1	0.001	0.032	0.8587
Rice fields	1	0.009	0.251	0.6169
Agro-forestry	1	0.084	2.243	0.1349
Shrubs	1	<0.001	0.008	0.9295
Inland Water Bodies	1	0.089	2.366	0.1247
Vineyards & orchards	1	0.013	0.334	0.5638
Coniferous	1	0.011	0.285	0.5935
Broadleaved & Mix forests	1	0.048	1.264	0.2615
Agriculture	1	0.045	1.188	0.2764
Altimetry	1	0.074	1.967	0.1615
Human population density	1	0.040	1.060	0.3039
Road network	1	0.017	0.462	0.4971
River network	1	0.004	0.114	0.7361
Average annual temperature	1	0.022	0.579	0.4471
Annual temperature range	1	0.003	0.091	0.7627
Annual rainfall	1	0.202	5.364	**0.0210**

NDVI	1	0.022	0.578	0.4475
Body size	1	0.017	0.442	0.5066
Body condition	1	0.147	3.914	**0.0485**
Error	424	0.038		

Table A2.2. Results obtained from GLMM analyses to examine gender, age, region and/or season variables as categorical predictors of body condition (residuals from the regression of total body weight against total body length) of Egyptian mongoose. District was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100 g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI) and body size (PCA1 of all measured biometrics and weight) were used as continuous predictors. [df – degrees of freedom; MS – mean square; F – statistic value; *p* – corresponding significance value. (*p* significant values at the 0.05 level are in bold).]

Effect	**df**	**MS**	**F**	***p***
Intercept	1	0.044	0.068	0.7951
District	11	1.110	1.692	0.0727
Region	1	1.555	2.369	0.1245
Season	3	2.280	3.474	**0.0161**
Gender	1	0.290	0.441	0.5068
Age	3	2.590	3.948	**0.0085**

Region×Season	3	0.593	0.904	0.4391
Region×Gender	1	1.253	1.910	0.1677
Region×Age	3	1.168	1.780	0.1502
Season×Gender	3	0.071	0.109	0.9551
Season×Age	9	1.164	1.774	0.0711
Gender×Age	3	0.168	0.256	0.8573
Region×Season×Gender	3	0.615	0.937	0.4227
Region×Season×Age	7	0.307	0.468	0.8576
Season×Gender×Age	9	0.413	0.630	0.7715
Region×Gender×Age	3	0.333	0.508	0.6772
Region×Season×Gender×Age	4	0.199	0.303	0.8757
Spleen weight	1	2.568	3.914	**0.0485**
Subcutaneous fat	1	5.812	8.857	**0.0031**
Visceral fat	1	5.131	7.819	**0.0054**
Egyptian mongoose abundance proxy	1	<0.001	0.001	0.9790
European rabbit abundance proxy	1	0.261	0.398	0.5284
Red-legged partridge abundance proxy	1	0.260	0.397	0.5291
Urban	1	0.474	0.722	0.3961
Rice fields	1	1.557	2.373	0.1242
Agro-forestry	1	<0.001	<0.001	0.9885
Shrubs	1	0.017	0.026	0.8724
Inland Water Bodies	1	0.312	0.476	0.4906
Vineyards & orchards	1	0.264	0.403	0.5259
Coniferous	1	0.114	0.174	0.6770
Broadleaved & Mix forests	1	0.681	1.037	0.3091
Agriculture	1	0.092	0.140	0.7087
Altimetry	1	0.120	0.182	0.6696

Human population density	1	0.192	0.293	0.5884
Road network	1	0.561	0.855	0.3556
River network	1	0.311	0.474	0.4915
Average Annual temperature	1	0.012	0.019	0.8906
Annual Temperature range	1	0.070	0.107	0.7436
Annual rainfall	1	0.087	0.132	0.7161
NDVI	1	0.001	0.002	0.9630
Body size	1	2.388	3.638	0.0571
Error	424	0.656		

APPENDIX A3

Table A3.1. Results obtained from GLMM analyses to examine gender, age, region and/or season variables as categorical predictors of **mammals'** food item of Egyptian mongoose. Province was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI), body size (PCA1 of biometrics and weight) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold)

Effect	**df**	**MS**	**F**	***p***
Intercept	1	1941.880	1.046	0.3105
Province	2	6374.162	3.435	**0.0388**
Region	0			
Season	3	1226.731	0.661	0.5793
Gender	1	352.549	0.190	0.6645
Age	3	3433.850	1.850	0.1479
Region x Season	3	1276.584	0.688	0.5630
Region x Gender	1	983.327	0.530	0.4695
Region x Age	3	2065.717	1.113	0.3510
Season x Gender	3	1321.471	0.712	0.5486
Season x Age	9	3498.310	1.885	0.0719
Gender x Age	3	948.589	0.511	0.6761
Spleen weight	1	84.519	0.046	0.8317

Subcutaneous fat	1	6319.246	3.405	0.0700
Visceral fat	1	550.892	0.297	0.5879
Egyptian mongoose abundance proxy	1	6266.647	3.377	0.0711
European rabbit abundance proxy	1	5966.743	3.215	0.0781
Red-legged partridge abundance proxy	1	6318.280	3.405	0.0700
Urban	1	635.080	0.342	0.5608
Rice fields	1	4384.266	2.363	0.1296
Agro-forestry	1	3682.634	1.985	0.1642
Shrubs	1	123.326	0.066	0.7975
Inland Water Bodies	1	7393.641	3.984	0.0505
Vineyards & orchards	1	2045.064	1.102	0.2981
Coniferous	1	259.193	0.140	0.7099
Broadleaved & Mix forests	1	3840.300	2.069	0.1556
Agriculture	1	4016.467	2.164	0.1466
Altimetry	1	307.189	0.166	0.6856
Human population density	1	118.696	0.064	0.8012
Road network	1	1854.114	0.999	0.3216
River network	1	614.280	0.331	0.5672
Average annual temperature	1	806.955	0.435	0.5122
Annual temperature range	1	924.370	0.498	0.4831
Annual rainfall	1	2007.538	1.082	0.3025
NDVI	1	46.642	0.025	0.8746
Body size	1	1708.114	0.920	0.3413
Body condition	1	7272.241	3.919	0.0524
Error	59	1855.668		

Table A3.2. Results obtained from GLMM analyses to examine gender, age, region and/or season variables as categorical predictors of **reptiles'** food item of Egyptian mongoose. Province was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI), body size (PCA1 of biometrics and weight) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold)

Effect	**df**	**MS**	**F**	***p***
Intercept	1	330.643	0.799	0.3750
Province	2	1110.984	2.685	0.0766
Region	0			
Season	3	769.860	1.860	0.1462
Gender	1	272.036	0.657	0.4207
Age	3	803.532	1.942	0.1327
Region x Season	3	379.492	0.917	0.4383
Region x Gender	1	70.611	0.171	0.6810
Region x Age	3	922.569	2.230	0.0942
Season x Gender	3	109.417	0.264	0.8508
Season x Age	9	596.537	1.442	0.1916
Gender x Age	3	588.412	1.422	0.2454
Spleen weight	1	237.881	0.575	0.4514

Subcutaneous fat	1	604.895	1.462	0.2315
Visceral fat	1	0.438	0.001	0.9742
Egyptian mongoose abundance proxy	1	502.517	1.214	0.2749
European rabbit abundance proxy	1	95.953	0.232	0.6319
Red-legged partridge abundance proxy	1	80.678	0.195	0.6604
Urban	1	8.884	0.021	0.8840
Rice fields	1	2285.156	5.522	**0.0221**
Agro-forestry	1	82.813	0.200	0.6563
Shrubs	1	88.062	0.213	0.6463
Inland Water Bodies	1	1300.796	3.144	0.0814
Vineyards & orchards	1	1059.308	2.560	0.1149
Coniferous	1	224.015	0.541	0.4648
Broadleaved & Mix forests	1	223.011	0.539	0.4658
Agriculture	1	70.670	0.171	0.6809
Altimetry	1	225.004	0.544	0.4638
Human population density	1	196.417	0.475	0.4935
Road network	1	145.076	0.351	0.5560
River network	1	299.765	0.724	0.3981
Average Annual temperature	1	406.057	0.981	0.3259
Annual Temperature range	1	846.879	2.047	0.1578
Annual rainfall	1	696.617	1.683	0.1995
NDVI	1	559.120	1.351	0.2498
Body size	1	563.415	1.362	0.2576
Body condition	1	540.740	1.307	0.2480

Error	59	413.800		

Table A3.3. Results obtained from GLMM analyses to examine gender, age, region and/or season variables as categorical predictors of **amphibians'** food item of Egyptian mongoose. Province was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI), body size (PCA1 of biometrics and weight) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold)

Effect	**df**	**MS**	**F**	***p***
Intercept	1	217.156	0.478	0.4919
Province	2	517.381	1.140	0.3268
Region	0			
Season	3	207.150	0.456	0.7138
Gender	1	11.449	0.025	0.8744
Age	3	459.303	1.012	0.3940
Region x Season	3	293.343	0.646	0.5884
Region x Gender	1	118.612	0.261	0.6111
Region x Age	3	156.005	0.344	0.7938
Season x Gender	3	411.819	0.907	0.4431

Season x Age	9	290.033	0.639	0.7592
Gender x Age	3	107.989	0.238	0.8696
Spleen weight	1	284.660	0.627	0.4316
Subcutaneous fat	1	190.982	0.421	0.5191
Visceral fat	1	961.474	2.118	0.1509
Egyptian mongoose abundance proxy	1	1104.596	2.434	0.1241
European rabbit abundance proxy	1	347.819	0.766	0.3849
Red-legged partridge abundance proxy	1	845.883	1.864	0.1774
Urban	1	60.683	0.134	0.7159
Rice fields	1	182.716	0.403	0.5282
Agro-forestry	1	1087.627	2.396	0.1270
Shrubs	1	1.117	0.002	0.9606
Inland Water Bodies	1	178.075	0.392	0.5335
Vineyards & orchards	1	32.861	0.072	0.7888
Coniferous	1	60.788	0.134	0.7157
Broadleaved & Mix forests	1	752.649	1.658	0.2029
Agriculture	1	1168.371	2.574	0.1140
Altimetry	1	67.684	0.149	0.7008
Human population density	1	25.046	0.055	0.8151
Road network	1	34.603	0.076	0.7834
River network	1	293.150	0.646	0.4248
Average Annual temperature	1	16.130	0.036	0.8511
Annual Temperature range	1	388.500	0.856	0.3587
Annual rainfall	1	1794.040	3.952	0.0515
NDVI	1	159.567	0.352	0.5555

Body size	1	555.983	1.225	0.2729
Body condition	1	94.394	0.208	0.6500
Error	59	453.912		

Table A3.4. Results obtained from GLMM analyses to examine gender, age, region and/or season variables as categorical predictors of **invertebrates'** food item of Egyptian mongoose. Province was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI), body size (PCA1 of biometrics and weight) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold)

Effect	**df**	**MS**	**F**	***p***
Intercept	1	3486.294	4.654	**0.0351**
Province	2	1393.965	1.861	0.1645
Region	0			
Season	3	405.449	0.541	0.6559
Gender	1	8.686	0.012	0.9146
Age	3	641.150	0.856	0.4690
Region x Season	3	732.160	0.978	0.4095
Region x Gender	1	105.037	0.140	0.7094

Region x Age	3	135.603	0.181	0.9089
Season x Gender	3	1555.482	2.077	0.1130
Season x Age	9	507.226	0.677	0.7262
Gender x Age	3	719.893	0.961	0.4172
Spleen weight	1	497.437	0.664	0.4184
Subcutaneous fat	1	533.907	0.713	0.4019
Visceral fat	1	558.392	0.746	0.3914
Egyptian mongoose abundance proxy	1	814.689	1.088	0.3012
European rabbit abundance proxy	1	1025.747	1.369	0.2466
Red-legged partridge abundance proxy	1	846.196	1.130	0.2922
Urban	1	436.157	0.582	0.4484
Rice fields	1	442.261	0.590	0.4453
Agro-forestry	1	133.343	0.178	0.6746
Shrubs	1	252.223	0.337	0.5639
Inland Water Bodies	1	55.218	0.074	0.7869
Vineyards & orchards	1	1666.509	2.225	0.1411
Coniferous	1	2319.203	3.096	0.0837
Broadleaved & Mix forests	1	1479.202	1.975	0.1652
Agriculture	1	490.862	0.655	0.4215
Altimetry	1	3302.574	4.409	**0.0400**
Human population density	1	2594.764	3.464	0.0677
Road network	1	2616.791	3.494	0.0666
River network	1	31.707	0.042	0.8377
Average Annual temperature	1	3964.053	5.292	**0.0250**
Annual Temperature range	1	2550.199	3.405	0.0700

Annual rainfall	1	255.725	0.341	0.5612
NDVI	1	214.106	0.286	0.5949
Body size	1	2301.698	3.073	0.0848
Body condition	1	5.450	0.007	0.9323
Error	59	749.006		

Table A3.5. Model selection, using AICc, for the effect of age, region, season, gender, region x age, region x gender, region x season, gender x age, gender x season, age x season, NDVI, spleen weight, body size and body condition on mammals, reptiles, amphibians and invertebrates food items of the Egyptian mongoose in Portugal. Rice fields (land use) was used in reptiles models only, annual rainfall in amphibians models only and altimetry and average annual temperature in invertebrates models only. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	**df**	**AICc**	**ΔAICc**	**AICc weight**
Mammals				
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	1038.92	0.00	0.64
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	1041.15	2.23	0.21

SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	1042.95	4.03	0.08
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	36	1043.37	4.45	0.07

Reptiles

SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	929.25	0.00	0.42
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	930.29	1.04	0.25
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	36	931.99	2.74	0.11
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	932.49	3.24	0.08
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	35	933.11	3.86	0.06
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	933.75	4.50	0.04

SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + RICE FIELDS + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	38	934.11	4.86	0.04

Amphibians

SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	915.48	0.00	0.33
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	916.01	0.53	0.25
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	916.75	1.27	0.17
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	35	918.38	2.90	0.08
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	36	918.81	3.33	0.06
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	35	919.62	4.15	0.04
SPLEEN WEIGHT + SEASON + AGE + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	35	919.89	4.42	0.04

SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	920.40	4.92	0.03
Invertebrates				
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	959.70	0.00	0.36
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	959.72	0.02	0.36
AVERAGE ANNUAL TEMPERATURE + SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	37	963.09	3.39	0.07
AVERAGE ANNUAL TEMPERATURE + SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	38	963.20	3.50	0.06
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	36	963.22	3.52	0.06
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + GENDER + BODY SIZE + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER	35	963.29	3.59	0.06

SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + GENDER + SEASON x AGE + SEASON x REGION + SEASON x GENDER + AGE x REGION + AGE x GENDER + REGION x GENDER	36	964.51	4.81	0.03

APPENDIX 4

Table A4.1. Results obtained from GLMM (Generalized Linear Mixed Models) analyses to examine age, region and/or season variables as categorical predictors of ovaries weight of Egyptian mongoose. Province was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold).

Effect	df	MS	F	*p*
Intercept	1	1.448	1.333	0.2495
Province	2	2.090	1.925	0.1484
Region	0			
Season	3	0.987	0.909	0.4377
Age	3	1.610	1.483	0.2202
Region*Season	3	0.477	0.440	0.7248
Region*Age	3	0.552	0.509	0.6768
Season*Age	9	1.027	0.946	0.4863
Region*Season*Age	5	1.368	1.260	0.2827
Spleen weight	1	0.707	0.651	0.4206
Subcutaneous fat	1	0.319	0.294	0.5882
Visceral fat	1	1.249	1.151	0.2846
Egyptian mongoose abundance proxy	1	0.519	0.478	0.4902

European rabbit abundance proxy	1	5.444	5.014	**0.0262**
Red-legged partridge abundance proxy	1	5.938	5.469	**0.0203**
Urban	1	1.614	1.487	0.2241
Rice fields	1	5.382	4.957	**0.0270**
Agro-forestry	1	0.360	0.331	0.5656
Shrubs	1	0.418	0.384	0.5357
Inland Water Bodies	1	0.172	0.159	0.6907
Vineyards & orchards	1	0.200	0.185	0.6680
Coniferous	1	1.470	1.354	0.2460
Broadleaved & Mix forests	1	0.496	0.457	0.4999
Agriculture	1	0.202	0.186	0.6664
Altimetry	1	2.419	2.228	0.1370
Human population density	1	0.273	0.252	0.6165
Road network	1	2.203	2.029	0.1558
River network	1	0.210	0.194	0.6602
Average annual temperature	1	1.615	1.487	0.2240
Annual temperature range	1	1.326	1.222	0.2703
Annual rainfall	1	0.512	0.471	0.4932
NDVI	1	0.755	0.696	0.4052
Body condition	1	1.928	1.776	0.1841
Error	212	1.086		

Table A4.2. Results obtained from GLMM (Generalized Linear Mixed Models) analyses to examine age, region and/or season variables as categorical predictors of testes weight of Egyptian mongoose. Province was used as random factor to control for spatial autocorrelation of samples. Spleen weight (expressed as g/100g body weight), subcutaneous and visceral fat, Egyptian mongoose, European rabbit and red-legged partridge abundances, land use (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous, broadleaved and mix forests, agriculture areas), altimetry, human population density, road network, river network, average annual temperature, annual temperature range, annual rainfall, Normalized Difference Vegetation Index (NDVI) and body condition (residuals from the regression of total body weight against total body length) were used as continuous predictors. (df – degrees of freedom; MS – Mean square; F – statistic value; *p* significant values are in bold).

Effect	df	MS	F	*p*
Intercept	1	1237.860	1.380	0.2417
Province	2	1009.170	1.125	0.3270
Region	0			
Season	3	2094.000	2.334	0.0756
Age	3	38440.950	42.845	**< 0.0001**
Region*Season	3	820.730	0.915	0.4350
Region*Age	3	2369.740	2.641	0.0509
Season*Age	9	1607.560	1.792	0.0725
Region*Season*Age	6	1203.790	1.342	0.2409
Spleen weight	1	205.760	0.229	0.6326
Subcutaneous fat	1	1747.920	1.948	0.1645
Visceral fat	1	836.370	0.932	0.3356
Egyptian mongoose abundance proxy	1	109.970	0.123	0.7267
European rabbit abundance proxy	1	248.740	0.277	0.5992

Red-legged partridge abundance proxy	1	948.090	1.057	0.3053
Urban	1	291.490	0.325	0.5694
Rice fields	1	4.980	0.006	0.9407
Agro-forestry	1	265.120	0.295	0.5874
Shrubs	1	866.610	0.966	0.3270
Inland Water Bodies	1	5.370	0.006	0.9384
Vineyards & orchards	1	752.210	0.838	0.3611
Coniferous	1	1595.830	1.779	0.1840
Broadleaved & Mix forests	1	88.810	0.099	0.7534
Agriculture	1	29.520	0.033	0.8563
Altimetry	1	670.820	0.748	0.3884
Human population density	1	159.520	0.178	0.6738
Road network	1	799.830	0.891	0.3463
River network	1	448.380	0.500	0.4805
Average annual temperature	1	894.640	0.997	0.3193
Annual temperature range	1	265.530	0.296	0.5871
Annual rainfall	1	113.620	0.127	0.7224
NDVI	1	156.280	0.174	0.6769
Body condition	1	5336.130	5.948	**0.0157**
Error	180	897.200		

Table A4.3. Model selection, using Akaike's Information Criterion (AICc), for the effect of age, region, season, region x age, region x season, age x season, NDVI, spleen weight, body condition on ovaries and testes weight (expressed as mg/100g body weight). [European rabbit and red-legged partridge abundances, and land use (rice fields) effects were used only for ovaries weight]. Province was used as random factor. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc that is considered the best model; df – degrees of freedom.

Models	**df**	**AICc**	**ΔAICc**	**AICc weight**
Ovaries weight				
SEASON + NDVI	7	805.40	0.00	0.19
SEASON + NDVI + RICE FIELDS	8	806.12	0.72	0.13
SEASON + RICE FIELDS	7	806.37	0.98	0.12
SEASON	6	806.69	1.30	0.10
SPLEEN WEIGHT + SEASON + NDVI	8	807.23	1.83	0.08
SPLEEN WEIGHT + SEASON + NDVI + RICE FIELDS	9	807.79	2.39	0.06
NDVI	4	807.95	2.55	0.05
SPLEEN WEIGHT + SEASON + RICE FIELDS	8	808.08	2.69	0.05
SEASON + NDVI + REGION	8	808.45	3.05	0.04
SPLEEN WEIGHT + SEASON	7	808.55	3.16	0.04
SPLEEN WEIGHT + NDVI	5	809.14	3.74	0.03
BODY CONDITION + SEASON + NDVI	8	809.41	4.01	0.03
SEASON + NDVI + REGION + RICE FIELDS	9	809.73	4.34	0.02
SEASON + REGION + RICE FIELDS	8	810.07	4.68	0.02
NDVI + REGION	5	810.28	4.88	0.02
BODY CONDITION + SEASON + NDVI + RICE FIELDS	9	810.31	4.91	0.02

SPLEEN WEIGHT + SEASON + NDVI + REGION	9	810.33	4.93	0.02
Testes weight				
SPLEEN WEIGHT + BODY CONDITION + SEASON + AGE + NDVI + REGION + SEASON x AGE + SEASON x REGION + AGE x REGION	28	2181.14	0.00	0.63
SPLEEN WEIGHT + SEASON + AGE + NDVI + REGION + SEASON x AGE + SEASON x REGION + AGE x REGION	27	2182.18	1.04	0.37

APPENDIX 5

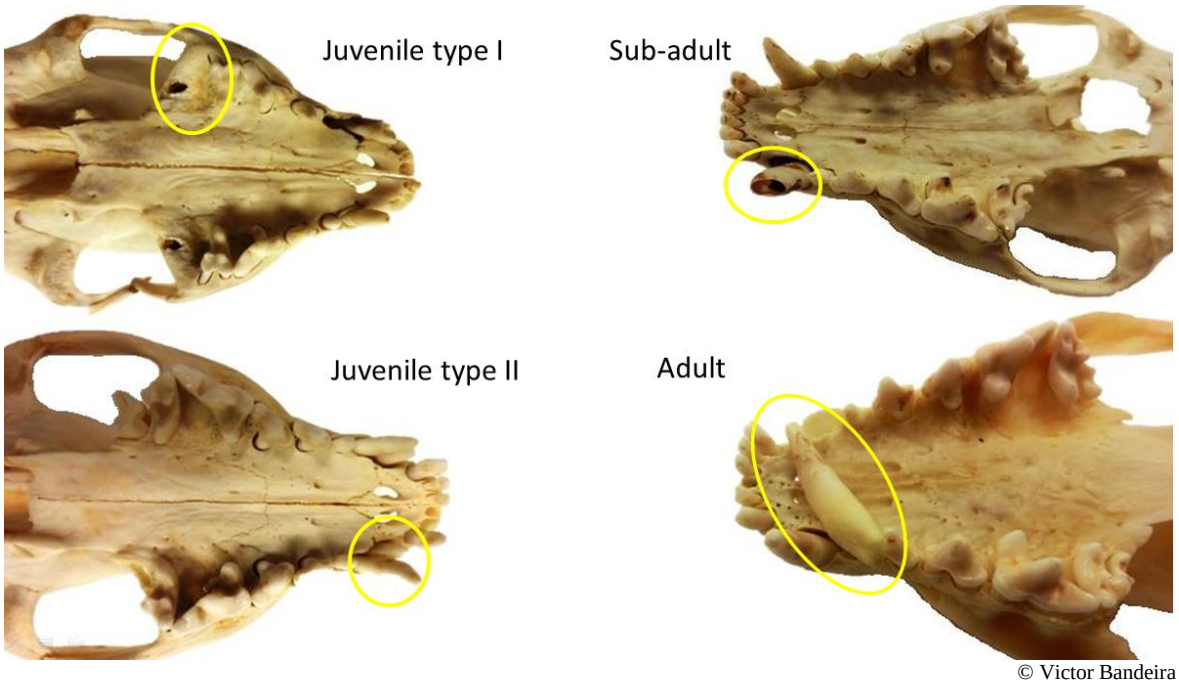


Fig. A5.1. Categorization of Egyptian mongoose's skulls using an age classification based on dental development analysis. (Juvenile type I: skull without molars and with only 32 teeth; Juvenile type II: skull with 40 teeth, but includes at least one milk tooth; Sub-adult: skull with 40 definitive teeth, but some still under development; Adult: skull with fully developed definitive teeth, with all the definitive teeth completely closed at its base)

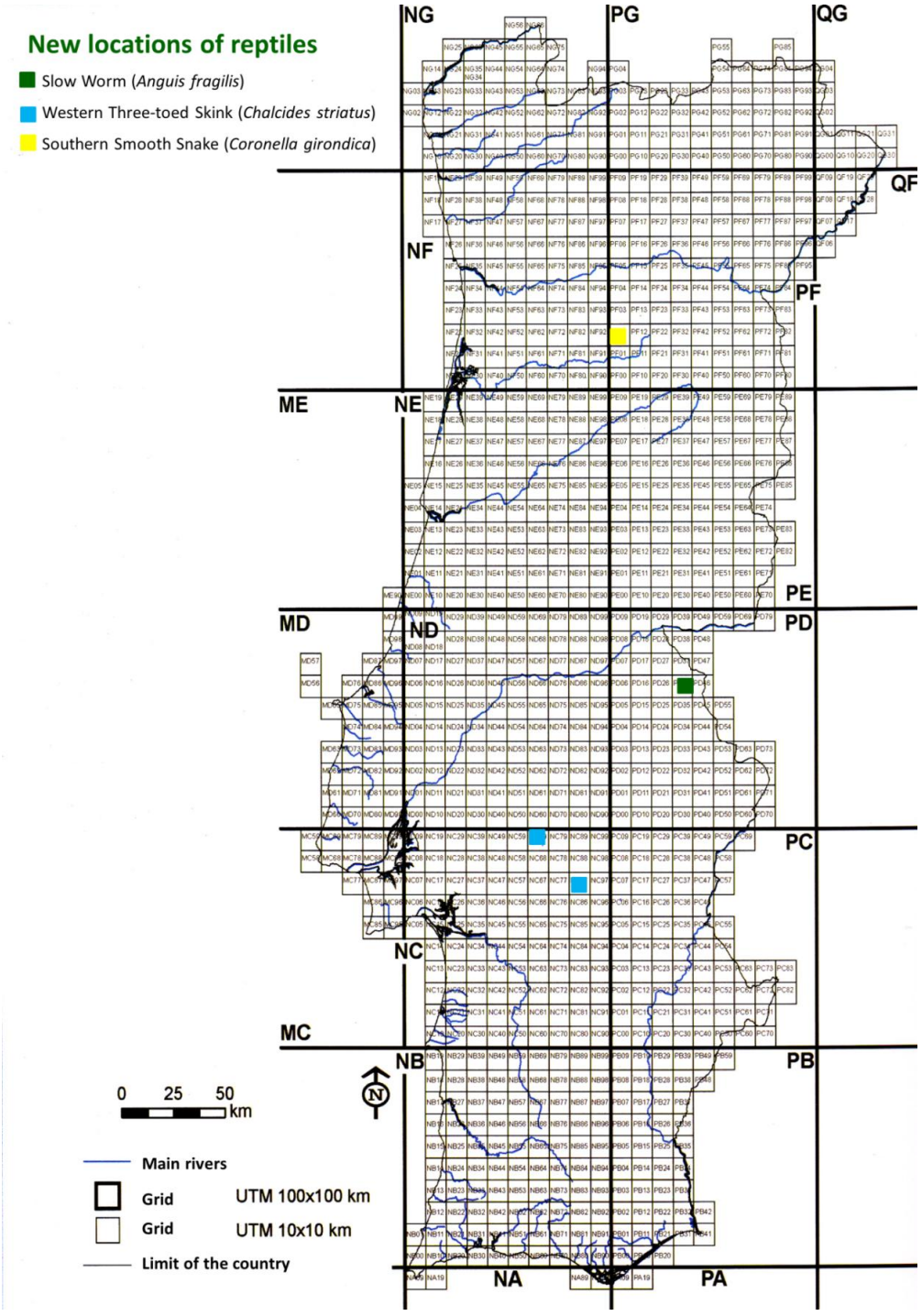


Fig. A5.2. New locations of reptiles in Portugal through Egyptian mongoose diet study.

[Map adapted from Loureiro, A., Ferrand de Almeida, N., Carretero, M.A. & Paulo, O.S. (Eds.) (2008). Atlas dos Anfíbios e Répteis de Portugal. Instituto da Conservação da Natureza e da Biodiversidade. Lisboa.]